

CLINICAL, BIOCHEMICAL AND IMMUNOLOGICAL
STUDIES OF FELINE THYROID FUNCTION

By

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ABSTRACT OF THESIS (Regulation 7.9)

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Double antibody radioimmunoassays for thyroxine (T4) and triiodothyronine (T3) were optimised and validated for use in cats. Serum total T4 concentrations of 318 and total T3 concentrations of 299 healthy cats were measured. In both sexes, T4 concentrations tended to decrease until five years of age and then rise again. For any given age, females and neutered females tended to have significantly higher T4 values than males and neutered males. Pedigree animals tended to have higher concentrations of T3 at any given age than cross-bred cats. Animals living in the same environment had significantly similar T4 and T3 concentrations respectively which, for T3 only, appeared to be due to a definite genetic component.

There was no obvious difference in the results of the T4 uptake test between euthyroid, hyperthyroid and hypothyroid cats. In normal feline serum, after agarose gel electrophoresis with tris-maleate buffer at pH 7.4, T4 was bound only to albumin and alpha-globulin, and feline T3 mainly to alpha-2-globulin and beta-globulin. In thyrotoxicosis, the percentages of T4 bound to albumin and alpha-globulin were decreased and increased respectively. These changes were not the result of hyperthyroxinaemia per se, nor to interference of T4 binding to albumin by non-esterified fatty acids. Changes in iodothyronine binding were seen in two euthyroid cats with elevated serum T3 and/or T4 concentrations, in two cats with euthyroid diseases, but not in two experimental hypothyroid animals.

The historical, clinical and diagnostic features of 74 hyperthyroid cats are presented. Approximately 70 per cent of cases had unilateral goitre. Surgical treatment for both unilateral and bilateral lobe involvement was effective but post-operative hypocalcaemia was common in the latter group.

Hypothyroidism was induced in two cats using ^{131}I and the resultant state monitored for 91 weeks. Both cats developed sub-normal rectal temperatures, reduced heart rates and bilaterally symmetrical alopecia and hyperpigmentation of the distal pinnae. Hyperkeratosis, myxoedema and apocrine gland changes were found in skin biopsies. Serum gamma-globulin concentrations increased progressively after thyroid ablation.

The historical and clinical features of 26 cases of feline endocrine alopecia (FEA) are described. A raised blood eosinophil count was highly reliable in distinguishing between FEA and physically similar conditions. Seventy-three per cent of cases responded totally to treatment with T3.

The thyroid stimulating hormone (TSH) stimulation test was optimised for cats. Six hours after TSH administration, cats with FEA had significantly lower, and absolute increases in, T4 concentrations than healthy cats. The serum T4 and T3 concentrations of the experimental hypothyroid cats failed to increase after TSH but a single hyperthyroid cat responded normally.

Thyroid and antinuclear antibodies were demonstrated in serum from ten and four of 29 hyperthyroid cats respectively, using the indirect immunofluorescence test with normal cat thyroid as substrate. Fifteen healthy control cats were negative for autoantibodies. Animals with strong positive results for thyroid antibodies were significantly more likely to have bilateral goitre with lymphocytic infiltrations than other thyrotoxic cats.

This thesis has been composed by me
and describes my own work except for
those matters which are specifically referred
to in the acknowledgements. It has not been
submitted in any form for another degree or
professional qualification.

Parts of the material which make up this
thesis have been published during the course of the study
and appear in the Appendix.

To my parents, who have been a
constant source of encouragement;
to my wife Liz, and my daughters
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ABBREVIATIONS

Abbreviations employed in the text are listed below.

The abbreviated term is cited in full when first used.

ABL	- Apicobasilar length
ACTH	- Adrenocorticotrophic hormone
ADP	- Adenosine diphosphate
ANA	- Antinuclear antibody
Anti-T3	- Antibodies to L-triiodothyronine
Anti-T4	- Antibodies to L-thyroxine
ANS	- 8-Anilino-1-napthalene-sulphonic acid
AP	- Alkaline phosphatase
AST	- Aspartate aminotransferase
ALT	- Alanine aminotransferase
ATP	- Adenosine triphosphate
Bo	- The per cent antibody bound in the zero standard
BUN	- Blood urea nitrogen
c-AMP	- Cyclic adenosine monophosphate
CCW	- Craniocaudal width
CK	- Creatinine kinase
CoA	- Coenzyme A
CPB	- Competitive protein binding
cpm	- Counts per minute
cv	- Coefficient of variation
DAS	- Donkey anti-sheep serum
df	- Degrees of freedom
DIT	- Diiodotyrosine

DNP	- 2,6-Diiodo-4-nitrophenol
ECG	- Electrocardiograph
EDTA	- Ethylenediamine tetraacetic acid
ESR	- Erythrocyte sedimentation rate
FEA	- Feline endocrine alopecia
Fig.	- Figure
FSH	- Follicle stimulating hormone
FT3	- Free L-triiodothyronine
FT4	- Free L-thyroxine
FT4I	- Free thyroxine index
G-6-P	- Glucose-6-phosphate
IgG	- Immunoglobulin G
IgM	- Immunoglobulin M
IRP	- International reference preparation
LDH	- Lactate dehydrogenase
LH	- Luteinising hormone
MIT	- Monoiodotyrosine
MMU	- Methimazole
n	- Number of observations
NAD	- Nicotinamide adenine dinucleotide
NADH	- Nicotinamide adenine dinucleotide (reduced)
NEFA	- Non-esterified fatty acids
NSS	- Non-immune sheep serum
PAS	- Periodic acid Schiff's
PBI	- Protein-bound iodine
PB ¹³¹ I	- Protein-bound ¹³¹ iodine
PB ¹³¹ I-CR	- Protein-bound ¹³¹ iodine conversion ratio
PBS	- Phosphate buffered saline

PCV	- Packed cell volume
PEG	- Polyethylene glycol
PTU	- Propylthiouracil
RBC	- Red blood cell
RIA	- Radioimmunoassay
RIU	- Radioiodine uptake
rpm	- Revolutions per minute
rT3	- Reverse triiodothyronine
SAPU	- Scottish Antibody Production Unit
sd	- Standard deviation
sem	- Standard error of the mean
T3	- L-Triiodothyronine
T3RIA	- L-Triiodothyronine by radioimmunoassay
T4	- L-Thyroxine
T4CPB	- L-Thyroxine by competitive protein binding
T4RIA	- L-Thyroxine by radioimmunoassay
TBG	- Thyroxine binding globulin
TBPA	- Thyroxine binding prealbumin
TRH	- Thyrotropin releasing hormone
TSH	- Thyroid stimulating hormone, thyrotropin

1. SUMMARY

The work described in this thesis is a study of a number of aspects of thyroid function in the domestic cat.

Chapter 2 provides an introduction to the subject and Chapter 3 reviews the literature on feline thyroid function.

DEVELOPMENT OF RADIOIMMUNOASSAYS FOR THYROID HORMONES IN FELINE SERUM (CHAPTERS 4-6)

In order to develop assays applicable to cats, conditions were first established for preparation of a "hormone-free" serum matrix. Endogenous thyroxine (T4) and triiodothyronine (T3) were successfully removed from normal cat serum by means of an anion exchange resin, and this serum was used as a base for the preparation of standards.

Radioimmunoassay (RIA) methods, using either solid phase antibodies or liquid phase double antibodies, were developed and optimised. However, the solid phase methods were found to be insufficiently sensitive, particularly for T3 and were not applied further.

SERUM THYROID HORMONE CONCENTRATIONS IN HEALTHY CATS (CHAPTER 7)

Serum total T4 and total T3 concentrations were measured in 318 and 299 healthy cats respectively, aged from four months to 13 years. Mean \pm sd concentrations were 26.1 ± 10.1 nmol/l for T4 and 0.69 ± 0.29 nmol/l for T3. Serum T4 and T3 concentrations of

individual animals were highly correlated. In both sexes, T4 concentrations tended to decrease until approximately five years of age and then rise again. A similar but less pronounced effect was found for T3. For any given age, females and neutered females tended to have significantly higher T4 values than males and neutered males, but these effects were not significant for T3. When other effects were accounted for, the effects of neutering were not significant for concentrations of either hormone. Pedigree animals tended to have higher concentrations of T3 at any given age than non-pedigree cats. Animals living in the same environment had significantly similar T4 and T3 concentrations. For T3, this appeared to be due to a definite genetic component but it was not possible to differentiate between environmental and genetic effects for T4. During this part of the study, two euthyroid cats with grossly elevated serum thyroid hormone concentrations were identified.

TRANSPORT OF THYROID HORMONES IN HEALTHY FELINE SERA (CHAPTER 8)

Techniques were developed to investigate the serum protein binding of T4 and T3 in healthy cats.

Initial studies with unfractionated sera using the T3 and T4 uptake tests showed less avid binding of both T3 and T4 in cats compared with man. Subsequently, agarose gel electrophoresis with tris-maleate buffer at physiological pH was used to define, more specifically, the nature of the serum iodothyronine binding in healthy cats. Feline T4 was bound to albumin (40.2 ± 6.7 per cent) and a protein with a mobility in the alpha-1/alpha-2-globulin

area (50.2 ± 6.5 per cent). Feline T3 was bound mainly in the alpha-2-globulin (37.1 ± 8.6 per cent) and beta-globulin (32.5 ± 6.3 per cent) zones, with smaller proportions being present in albumin and alpha-1-globulin (figures are mean values \pm sds). T3 was also present in the gamma-globulin zone but the technique did not prove whether or not this was protein bound. There were major differences in T4 and T3 binding between cats and humans.

TRANSPORT OF THYROID HORMONES IN FELINE SERA WITH ABNORMAL TOTAL THYROXINE AND TRIIODOTHYRONINE CONCENTRATIONS (CHAPTER 9)

The same techniques were used to investigate serum thyroid hormone protein binding in cats with abnormal total T4 and T3 concentrations.

The T4 uptake test was unchanged in feline hyperthyroidism and hypothyroidism and is therefore of no value in the diagnosis of these conditions. In thyrotoxicosis, the percentage of T4 bound to albumin was decreased and the percentage bound to alpha-globulin, increased. These changes were not the result of hyperthyroxinaemia per se, nor, in the small number of cases studied, were they due to blockage of binding of T4 to albumin by non-esterified fatty acids. In hyperthyroid cats, there were also small but significant percentage decreases of T3 bound to albumin and alpha-1-globulin. After successful treatment of the condition, T4 and T3 binding tended to revert to that seen in euthyroid animals. Two cats in which hypothyroidism had been induced using radioiodine showed no abnormalities of thyroid hormone binding. However, there were T3

and/or T4 binding changes in two euthyroid cats with hypertriiodothyroninaemia and/or hyperthyroxinaemia and in two cats with euthyroid diseases.

FELINE HYPERTHYROIDISM (CHAPTER 10)

A series of 74 hyperthyroid cats is described.

The historical and clinical features and the results of investigative procedures (radiography, electrocardiography, haematology and biochemistry) agreed closely with other series. Pre-surgical treatment using propranolol and potassium iodide was effective in reducing elevated heart rates and technical problems at surgery but not in producing a return to the euthyroid state pre-operatively. Seventy per cent of cases were judged clinically to have unilateral thyroid lobe disease. Both unilateral and bilateral cases responded well to surgery but post-operative hypocalcaemia was common in the latter group.

EXPERIMENTALLY-INDUCED FELINE HYPOTHYROIDISM (CHAPTER 11)

Because a case of naturally-occurring, acquired, feline hypothyroidism has never been confirmed, the condition was induced experimentally and the resultant clinical and laboratory changes monitored over a 91 week period.

After radioisotope administration, the serum T4 and T3 concentrations of both animals were consistently below the detection limits of the respective assays and both animals had sub-normal rectal temperatures and reduced heart rates. Widespread hair loss

did not develop but an unusual dermatosis, characterised by alopecia and hyperkeratosis, occurred over the distal halves of the pinnae. Hyperkeratosis, myxoedema and reduced numbers of apocrine glands were found histologically in skin biopsies from these and other sites. Serum cholesterol concentrations remained in the normal range but there was a progressive increase in serum gamma-globulin and total protein concentrations.

THYROID FUNCTION IN FELINE ENDOCRINE ALOPECIA (CHAPTER 12)

A series of 26 cases of feline endocrine alopecia (FEA) is described.

The diagnosis was made on the history, results of physical examination, negative laboratory findings for diseases with similar presenting signs and failure to respond to flea control measures. A raised eosinophil count was highly reliable in distinguishing between FEA and physically similar conditions. Although clinical and basal laboratory data did not suggest hypothyroidism as the cause of FEA, 73 per cent of cases responded totally to treatment with liothyronine sodium.

THE THYROID STIMULATING HORMONE STIMULATION TEST IN THE ASSESSMENT OF FELINE THYROID FUNCTION (CHAPTER 13)

The optimisation of the thyroid stimulating hormone (TSH) stimulation test in healthy cats is described.

Cats with FEA had lower total T4 concentrations and absolute increases in T4 concentrations six hours after TSH administration

than did healthy cats but these values were not significantly different for T3. Some cases of FEA may be a manifestation of low thyroid reserve. The two hypothyroid cats failed to show any increase in serum T4 or T3 concentrations after administration of TSH but a single case of hyperthyroidism responded normally.

AUTOANTIBODY STUDIES IN FELINE NATURALLY-OCCURRING HYPERTHYROIDISM AND RADIOIODINE-INDUCED HYPOTHYROIDISM (CHAPTER 14)

Indirect immunofluorescence studies were carried out in cases of feline thyrotoxicosis and experimental hypothyroidism.

Thyroid autoantibodies were demonstrated in sera from ten of 29 hyperthyroid cats and antinuclear antibody was found in a further four cases. Of the five sera showing a strong reaction for autoantibodies, four had bilateral goitre and lymphocytic infiltrates were found in four excised thyroid glands. During follow-up periods of three to 40 months, three cats developed recurrent hyperthyroidism. Two cats had high titres of thyroid antibodies and bilateral goitre with lymphocytic infiltrates, while the remaining case was antinuclear antibody positive and had unilateral goitre with lymphocytes. Autoantibodies were not demonstrated in the two cases of experimental hypothyroidism.

2. INTRODUCTION

Although details of feline thyroid anatomy date from the end of the last century, until recently, comparatively little was known about feline thyroid physiology. This may have resulted, in part, from an apparent lack of thyroid-related diseases in cats. Thus, the earliest investigations into normal feline thyroid function, measuring protein-bound iodine, were actually carried out by medical research workers who required control values to compare with results obtained after the experimental production of feline hyper and hypothyroidism. As other techniques were developed for use in human medicine, occasional reports of their use in cats appeared in the literature. However, these often involved very small numbers of animals and were undertaken simply to contrast with values obtained in other species.

The possible repercussions of changes in serum protein binding of thyroxine (T4) and triiodothyronine (T3) were quickly recognised in human medicine, and medical endocrinologists carried out the earliest studies into iodothyronine transport in cats for comparative purposes. Although there were qualitative and quantitative differences in binding, a protein comparable with human thyroxine binding globulin (TBG) appeared to be present in feline serum. However, while some veterinary research workers subsequently came to similar conclusions, others failed to identify a feline TBG. The nature of thyroid hormone protein binding in the species has never been properly addressed and consequently, conditions of abnormal iodothyronine transport, so well-documented in man, have never been recorded in cats.

The first disease of cats to be linked with abnormalities of thyroid function was feline endocrine alopecia (FEA). A number of authors have reported that the condition responds to thyroid hormone supplementation but others have stated that affected cats are not hypothyroid, although only one paper has included supportive data. While attempts have been made to induce hypothyroidism experimentally in cats, modern tests of thyroid function were not used to confirm the condition and clinical changes were poorly recorded. A case of naturally-occurring, acquired, feline hypothyroidism has never been definitively diagnosed.

The first detailed reports of feline hyperthyroidism were published in 1980, after the present study had begun. In fact, two short reports of cases had been published in 1979 in North American conference proceedings but these were not readily available in the U.K. It quickly became obvious that hyperthyroidism was a common disease of older cats and this provided the incentive for further investigations into normal feline thyroid physiology. However, subsequent reports of plasma T4 and T3 concentrations in healthy cats have merely provided details of ranges without studying the possible effects of a number of variables.

It was against this background that the present study was begun. Its aims were to:

- (i) develop RIAs for T4 and T3 which would accurately and precisely measure these analytes in feline serum.
- (ii) use these assays to determine the normal ranges for T4 and T3 and to identify any effects of breed, sex, age, environment or heredity.

- (iii) develop techniques to determine the normal mechanisms of T4 and T3 transport in feline serum.
- (iv) identify whether hyperthyroidism and hypothyroidism occurred naturally in cats by using the developed techniques to investigate clinically suggestive cases.
- (v) induce hypothyroidism experimentally in cats and monitor its progression by modern clinical and laboratory methods.
- (vi) determine whether FEA might be a manifestation of hypothyroidism.
- (vii) optimise the thyroid stimulating hormone stimulation test for use in cats and to determine its value in the investigation of possible feline thyroid diseases.
- (viii) conduct such other studies as might be indicated in the light of observations made during these investigations.

3. THYROID FUNCTION IN THE CAT - REVIEW OF THE LITERATURE

3.1 HISTORICAL ASPECTS

Excellent accounts of the history pertaining to the thyroid gland of man and animals have been given by Rolleston (1936) and Werner (1978a), whose summaries form the basis of this review.

The thyroid was described by Galen in his *De Voce* some time between A.D. 130 and 200. He considered that it provided a lubricating fluid to the larynx and its cartilages to facilitate speech. Vesalius (1543) was the first to provide a fuller account of the gland. Eustachius (1520-1575, cited 1714) called it the "glandula laryngea" and applied the term "isthmus" to the part connecting the two lobes. However, it was not until 1656 that Wharton named the organ "the thyroid" on the basis of shape, from the Greek "thyreos" meaning "an oblong shield" plus "eidos" meaning "form". He wrote: "It contributes much to the rotundity and beauty of the neck, filling up the vacant spaces round the larynx and making its protruberant parts almost to subside, and become smooth, particularly in females which renders their necks more even and beautiful".

Cowper (1698) concluded that the thyroid had the same function as the thymus which he regarded as a lymphatic gland for the upper part of the body. Vercellone (1711) thought that the thyroid was a bag of worms, the ova of which mixed with the chyle and passed by ducts into the oesophagus. Parry (1825) felt that the thyroid was a "vascular shunt" cushioning the brain against sudden increases in blood flow.

By means of experimental surgical thyroidectomy, Cooper (1827) studied the relationship between the thyroid and various body functions and subsequently King (1836) reported that the gland had an internal secretory function. Reverdin and Reverdin (1883) associated naturally-occurring myxoedema with the state post-thyroidectomy, but it required Semon (cited by Rolleston, 1936) and Horsley (1885) to confirm the connection. Because the parathyroids were only recognised by Gley in 1891, death usually followed thyroidectomy and it was not until 1896 that Vassale and Generali separated myxoedema following thyroidectomy from hypocalcaemic tetany. The specific role of the parathyroids was confirmed in 1898 when Welsh carried out parathyroidectomy without thyroidectomy in the cat and in 1909, McCallum and Voegtlin demonstrated that hypocalcaemia followed parathyroidectomy.

Baumann (1895) made the association between iodine and thyroid function when he discovered a high concentration within the gland and Oswald (1899) confirmed this with his work on thyroglobulin. In 1900, Gley and Bourcet identified the presence of organic iodine in plasma in combination with plasma proteins. In 1915, Kendall isolated L-3,5,3¹,5¹-tetraiodothyronine (L-thyroxine, T₄) from thyroid tissue and its chemical structure was identified by Harington (1926) and Harington and Barger (1927). Thyroid stimulating hormone, thyrotropin (TSH) was discovered by Smith and Smith (1922) who found that extracts of fresh bovine pituitary glands would reactivate the atrophied thyroid glands of hypophysectomised tadpoles.

A further major discovery was made with the identification of an iodine-containing substance other than T₄ in animal plasma

(Gross and Leblond, 1951 a and b) and in human plasma (Gross and Pitt-Rivers, 1951). This was shown to be L-3,5,3¹-triiodothyronine (T3) by Gross and Pitt-Rivers (1952). This compound proved to be more potent and rapid in onset than T4 and these workers speculated that T4 was the form in which thyroid hormone was secreted while T3 was the form that was active in tissues. In 1952, Gordon, Gross, O'Connor and Pitt-Rivers reported that plasma T4 was largely associated with a single protein constituent, thyroxine binding globulin (TBG).

L-3,3¹,5¹-triiodothyronine (reverse T3, rT3) was identified in the blood and thyroglobulin of the rat by Roche, Michael and Nunez in 1956.

The hypothalamic-pituitary regulation of thyroid hormone production has been extensively reviewed by Burger and Patel (1977). The identification of a hypothalamic TSH-releasing factor was claimed by Shibusawa, Saito, Nishi, Yamamoto, Tomizawa and Abe (1956) but this work did not receive wide acceptance. The first incontrovertible evidence for the existence and purification of thyrotropin releasing hormone (TRH) was published by Guillemin, Yamazaki, Jutisz and Sakiz in 1962 and its chemical structure was determined by Schally, Arimura, Bowers, Wakabayashi, Kastin, Redding, Mittler, Nair, Pizzolato and Segal (1970).

Baber (1876) in his work with dogs was the first researcher to note two kinds of thyroid cells. Copp, Davidson and Cheney (1961) and Copp, Cameron, Cheney, Davidson and Henze (1962) reported their discovery of calcitonin and subsequently Foster, MacIntyre and Pearse (1964) proposed that this compound was produced by one of the types of thyroid cells. In 1965, Gudmundsson, MacIntyre and Soliman

synthesised calcitonin. The cells that produced it endogenously were named C cells by Pearse in 1966.

3.2 EMBRYOLOGICAL DEVELOPMENT OF THE THYROPARATHYROID APPARATUS

Few studies have been carried out on the embryological development of the feline thyroid. Thomas and Nathanielsz (1980) stated that the process is essentially the same in all mammalian species. Thus, the thyroid glandular epithelium arises from the entodermal lining of the foregut at the level of the first pair of pharyngeal pouches. The gland first appears as a distinct entodermal pocket, the thyroid diverticulum. This ventral outgrowth from the middle of the foregut remains, for some time, attached by a narrow neck, the thyroglossal duct. As development proceeds, this duct becomes a solid stalk of cells and eventually breaks off, leaving only a surface indentation on the back of the tongue, the foramen caecum. The thyroid soon becomes a bilobed, solid mass of cells when set free from the atrophied stalk. The connective tissue capsule and vascular interstitial tissue are derived from surrounding mesenchyme (Venzke, 1975).

Winiwarter (1935) noted changes in the pace of feline foetal thyroid development. During the first half of gestation, until the first vesicles of colloid appeared, growth was slow ("embryonic") but there was then a rapid multiplication of follicles and a phase of activity, followed by an almost complete resting phase until after birth.

Ramsay (1938) carried out an extensive study of the development of feline thyroid parenchyma using the entire Cornell collection of

cat embryos stained with either Mayer's HCl carmine or with haematoxylin and eosin, and a graded series of fetuses stained with haematoxylin and eosin. He divided the process arbitrarily into five: the plate stage, diverticulum formation, cavity formation, the prefollicular stage and the follicular stage.

In the plate stage, the youngest embryo (11 somites) showed no definite area for thyroid formation, the future thyroid plate being discernible only by its position and the absence of mesenchyme between it and the underlying aortic sac. The first indication of a delimitation in the thyroid area occurred at the 16 to 18 somite stage with a slight depression in the pharyngeal floor immediately over the aortic sac and between the ventral grooves of the first pair of pharyngeal pouches. Embryos of 22 and 23 somites possessed easily recognised thyroid plates showing both thyroid pits and a massive increase in mitotic figures. This active growth phase initiated diverticulum formation and tended to fill up the thyroid pit. At the same time, the mesenchyme below the floor of the pharynx increased and migrated between the plate and the aortic sac. The thickened plate was countersunk by the continued growth of the mesenchyme and its area of attachment to the pharyngeal epithelium was markedly constricted, thus changing the plate into a diverticulum.

There was considerable variation between embryos but newly formed diverticula were usually saccular. Due to growth of the thyroid epithelium, the walls of the diverticulum became evaginated, producing secondary diverticula (primitive thyroid follicles) as well as solid buds and sprouts of cells from the original saccular anlage. This was the initial stage of cavity formation. Crowding

and sloughing of cells caused the cavities of the hollow buds thus formed to lose their connection with the main cavity very rapidly. The residual lumen was lost by the production of a massive portion of thyroid cells in the midline. To establish a more extensive contact with the surrounding vascular mesenchymal bed, the thyroid cells of 6mm to 8mm embryos separated to form tiny intercellular clefts (the intraglandular clefts of Norris). These opened at the outside of the anlage and were invaded by mesenchyme which ultimately reduced the central massed region of the thyroid to plates and bands two cells in thickness.

In the prefollicular stage, Complex IV fused with the thyroid anlage (12mm to 13mm embryonic length). Primitive follicles occurred most numerous in the lateral lobes while the central region (isthmus) contained few. The walls of the primitive follicles thickened but only the single layer of cells around the lumen showed any definite arrangement. By the 40mm stage, the gland was composed of irregularly branching bands and buds of compact, highly vascularised thyroid parenchyma and numerous primitive follicles. The follicular stage was marked by the appearance of a highly refractile, acid-staining substance in the primitive follicles (40mm to 41mm foetal length). By 50mm, this colloid had increased in amount and these structures were termed "primary thyroid follicles". Initially, the cells surrounding the colloid had no orientation but as the globules increased in size, the adjacent cells became typically arranged and the characteristic picture of adult thyroid parenchyma emerged. Secondary follicles were formed by constriction or division of primitive and primary follicles and by direct hollow outgrowths from existing follicles followed by constriction.

However, some of the thyroid parenchyma retained a marked tendency for growth and continued to add thyroid material for some time following birth.

The authors drew particular attention to two features described. Firstly, the development of primitive follicles directly from the open thyroid diverticula appeared to be a process peculiar to the cat. Secondly, primary feline thyroid follicles produced colloid before they possessed lumina, whereas the reverse occurred in man (Kingsbury, 1915; Norris, 1916).

Latimer (1939a) weighed the thyroids of 229 foetal cats. The glands were relatively heaviest in the smallest foetuses, decreasing rapidly in percentage weight at first, and then more slowly to birth.

The early growth and development of the thyroid does not appear to be TSH dependent since histological development, colloid formation and storage can occur in the absence of a pituitary (Thomas and Nathanielsz, 1980).

In the cat, as in other species, a portion of the thyroglossal duct may persist post-natally and form a cyst due to proteinaceous material secreted by the lining epithelium (Martin and Capen, 1983).

The parathyroids develop separately from the dorsal diverticula of the third and fourth branchial pouches. Each diverticulum forms into a solid mass of cells of entodermal origin, to become differentiated into two pairs of globular glands which become detached from the pharyngeal mucosa. The pair derived from the third branchial pouch are termed parathyroids III or the cranial or external glands and those derived from the fourth branchial pouch, parathyroids IV or the caudal or internal parathyroids (Kohn, 1895).

3.3 ANATOMY OF THE THYROPARATHYROID APPARATUS

Gross anatomy

Kohn (1895) was the first person to detail the anatomy of the thyroid of the cat, since when there have been numerous descriptions published. It is generally regarded by anatomists to be a single gland consisting of two lobes (Holzworth, Theran, Carpenter, Harpster and Todoroff, 1980). Embryologically, the lobes are connected by an isthmus but this is not usually present in adults (Nicholas and Swingle, 1924a; Andrew, 1959; Lucke, 1964; Walker, 1967; McDonald, 1975, 1980; Schaer, 1981), although a large lymph channel marks its foetal location (Ramsay and Bennett, 1943). However, Nickel, Schummer and Seiferle (1975) state that the lobes are usually joined by a 1mm to 2mm wide isthmus. When present, the isthmus may be glandular (Lucke, 1964) or fibrous (Holzworth et al., 1980) connecting the lobes ventrally across the trachea (Field and Taylor, 1969; Kaneko, 1970, 1980a) by their inferior poles (Nicholas and Swingle, 1924a). The lobes are flattened, long ellipses, generally with rounded anterior and pointed posterior edges (Lucke, 1964), although a thin, tongue shape has also been described (Winiwarter, 1935).

Carlson (1914) was of the opinion that normal cats' thyroids were constant in size. Bustad and Fuller (1970), however, writing of mammals generally (in which they included the cat), commented that thyroid size fluctuated markedly with advancing age, changes in physiological state such as pregnancy and lactation, and in response to environmental factors such as stress, temperature and diet (particularly iodine content). Clark and Meier (1958) agreed that adult feline thyroid size depended upon age. In mature animals,

each lobe is approximately 2cm long, 0.5 to 0.6cm deep and 0.3cm wide (Daniel, Gale and Pratt, 1962; Lucke, 1964) with a colour variably described as pale to medium mahogany (Lucke, 1964), yellow or red-brown (Holzworth et al., 1980), light brownish-yellow (Black and Peterson, 1983) and tan (Peterson, Birchard and Mehlhaff, 1984).

The thyroid lobes lie within the cervical fascia, between the sternothyroideus muscles and the trachea, with their anterior poles just below the cricoid cartilage and extending downwards over the first five to six tracheal rings (Nicholas and Swingle, 1924a; Lucke, 1964; Crouch, 1969; Bustad and Fuller, 1970; Martin and Capen, 1983; Birchard, Peterson and Jacobson, 1984; Peterson, Birchard and Mehlhaff, 1984). Contrary to all other authors, Nickel, Schummer and Seiferle (1975) described the lobes as lying in the area of the seventh to tenth tracheal rings. Dorsally, the lobes are in close proximity to the carotid sheath and the vago-sympathetic trunk. The right recurrent laryngeal nerve is medial to the gland and courses along the trachea's right dorsolateral surface. The left recurrent laryngeal nerve lies between the trachea and the oesophagus (Black and Peterson, 1983). A thick, firm, fibrous capsule surrounds each lobe (Ramsay and Bennett, 1943; Soderberg, 1958; Black and Peterson, 1983). Fibro-elastic connective tissue septa extend from the capsule into the glandular parenchyma providing an internal support, transporting blood vessels, lymphatics and nerves and separating the gland into indistinct lobules. There are fewer connections between the parenchyma and the capsule in young animals, the increase with age possibly being due to secondary adhesion formation (Ramsay and Bennett, 1943).

Brody and Kibler (1941) noted that, in general, in mature domestic animals (in which they included the cat), the thyroid gland represented 100mg/kg of bodyweight and proportionately more in the young. Carlson (1914) examined 20 healthy, pregnant queens with a mean bodyweight of 3.0kg (range 2.81kg to 4.6kg) and found their mean thyroid weight to be 0.25g (range 0.2g to 0.3g) thus giving a mean bodyweight/thyroid weight ratio of 12,000:1 (range 8,800:1 to 16,200:1). Eighty-five kittens taken from these queens either before term or at birth had a mean bodyweight of 80g (range 41g to 125g), a mean thyroid weight of 0.017g (range 0.012g to 0.022g) with a resultant mean ratio of 4,705:1 (range 4,500:1 to 7,300:1). There was no evidence that thyroid weight increased or decreased proportionately with a gain or loss of body fat.

Latimer (1939a) examined the thyroid glands of 35 newborn kittens and 104 adult cats. At birth, the gland represented 0.02 per cent of bodyweight but this fell to 0.0084 per cent in mature animals. In adults, there was no significant sex difference in either absolute or percentage weight, with mean weight for males being 0.235g and for females 0.218g (Latimer, 1939b).

Knigge (1961) examined the thyroids of ten healthy cats and reported that the two lobes may vary in weight from 0 to 29.6 per cent (mean \pm sd was 12.3 ± 6.5 per cent) with neither lobe consistently weighing more than the other. The mean weight of the two lobes of a further 65 cats was $248\text{mg} \pm 24\text{mg}$ (range 101mg to 398mg) with a mean relationship to bodyweight of $75.9 \pm 6.2\text{mg/kg}$. Daniel, Gale and Pratt (1962) reported that each feline thyroid lobe weighed 0.2g to 0.5g, whereas Nickel, Schummer and Seiferle (1975) stated that the absolute weight of the whole gland varied between 0.19 and 1.45g but

in neither case were further details given. None of these workers apparently removed parathyroid tissue from the excised glands and thus the above figures presumably indicate combined thyroid/parathyroid weights.

Kohn (1895) gave the first description of the cat's parathyroid glands. One external (cranial) and internal (caudal) gland is normally associated with each thyroid lobe. However, the number of each is variable, particularly in the case of the caudal glands (Nicholas and Swingle, 1924a).

The external parathyroids are usually located near the cranial pole of each lobe in fascia external to the thyroid capsule (Nicholas and Swingle, 1924b; Black and Peterson, 1983; Martin and Capen, 1983; Birchard, Peterson and Jacobson, 1984; Peterson, Birchard and Mehlhaff, 1984). Their position is not, however, constant and they may be found in practically any site on the anterior or ventral surfaces of the glands (Nicholas and Swingle, 1924a; Holzworth et al., 1980). They are spherical, 2mm to 7mm in diameter (Nicholas and Swingle, 1924a; Holzworth et al., 1980; Black and Peterson, 1983) and tan (Holzworth et al., 1980) or pink (Black and Peterson, 1983) in colour.

The internal glands are more variable in position than the externals and although they commonly lie within the thyroid parenchyma at the caudal pole of each lobe (Nicholas and Swingle, 1924a; Holzworth et al., 1980; Black and Peterson, 1983; Birchard, Peterson and Jacobson, 1984; Peterson, Birchard and Mehlhaff 1984), they may be found anywhere on the tracheal surface of the lobe (Nicholas and Swingle, 1924a) or within the thyroid capsule (Martin and Capen, 1983). The internal glands are smaller than the

externals and spherical, although a star shape has also been described (Winiwarter, 1935).

Walker (1967) stated that "two pairs of parathyroid glands are embedded in the dorsomedial surface of the thyroid but cannot be seen grossly". In their description of the feline parathyroid glands, Field and Taylor (1969) stated that they "are located on the dorsal side of the thyroid gland near its anterior end". They were presumably referring to the external glands and made no mention of the internals.

There is an intimate relationship between the thymus and the thyroid gland of the cat. In many cases, a portion of the cervical thymus is found attached to, or even partially embedded in, the thyroid lobes. In adults, the cervical thymus tissue may be lacking or replaced by fat but in younger animals, some thymus is generally present in close connection with the thyroid and external parathyroids (Kohn, 1895).

Vascularisation

According to McDonald (1975, 1980), the vascular supply to the (vertebrate) thyroid fluctuates considerably depending on the activity of the gland. Considering its size, it receives one of the richest supplies of blood of any organ with the exception of the adrenal. Soderberg (1958) was able to show the rapid, short-term changes in blood flow from the feline thyroid in response to a number of nervous, hormonal and chemical stimuli.

Nicholas and Swingle (1924a) carried out an extensive investigation into the vascularisation of the cat's thyroparathyroid apparatus using one of two methods. In some animals, chrome yellow was injected through the femoral artery immediately after death, to

delineate the main arterial circulation. In others, ammoniacal carmine solution was injected into the inferior vena cava of anaesthetised animals. The abdominal aorta was then severed and clamped to permit a slight blood flow from the proximal end and when the heart action ceased, pressure was applied by syringe. The thyroparathyroid apparatus was then placed in a mixture of ten per cent formalin and five per cent acetic acid and cleared in Spalteholz's fluid (50 per cent benzol benzoate and 50 per cent methyl salicylate). This technique reveals the thyroid tissue as pink, the parathyroids white and the vasculature red. The authors found that the arterial supply enters each thyroid lobe at its cranial pole and corresponds to the cranial or superior thyroid artery of other species. Immediately after entering the thyroid, it gives off several small branches, which supply the gland plus both parathyroids. The cranial thyroid arteries branch from the common carotid artery opposite the larynx, from where they run caudally to each thyroid lobe (Schaer, 1981; Birchard, Peterson and Jacobson, 1984; Peterson, Birchard and Mehlhaff, 1984). The caudal or inferior thyroid artery is not present in most cats (Nicholas and Swingle, 1924a).

Venous drainage from the thyroid and parathyroid glands is usually described as being via the cranial and caudal thyroid veins (Black and Peterson, 1983; Birchard, Peterson and Jacobson, 1984; Peterson, Birchard and Mehlhaff, 1984), the latter corresponding to the v. thyroidea media as described by Schummer, Wilkens, Vollmerhaus and Habermehl (1981a). Whatever the terminology, most of the venous outflow follows these caudal veins which leave the caudal border of each lobe (Soderberg, 1958; Schaer, 1981). Thence,

there is acknowledged to be some variation, with some authors stating that these vessels form a single vein in most cats which runs down the ventral surface of the trachea, emptying into the right or left jugular vein (Nicholas and Swingle, 1924a; Soderberg, 1958), while others describe the caudal veins continuing to run separately, eventually joining the respective internal jugular veins (Schaer, 1981). The cranial vein is a small vessel which arises near the cranial pole of each thyroid lobe and drains into the internal jugular vein at that position (Schaer, 1981; Schummer et al., 1981a).

Schummer et al. (1981b) alone describe a vessel they term the v. thyroidea caudalis which is not the counterpart of the caudal thyroid vein described above. This unpaired structure arises from the cranial thyroid vein usually draining only the left thyroid lobe together with the trachea and the sternohyoid and sternothyroid muscles. It runs ventrally along the neck between these muscle groups and enters the left, or rarely the right, brachiocephalic vein.

Lymphatic drainage

Ramsay (1938) reported that the mature cat's thyroid possessed an almost complete lymph sac surrounding the entire thyroid except at the point of entrance and exit of blood vessels. The lymphatic endothelium penetrated the lobules to such an extent that each follicle was in close contact with it. He also made brief comments about the chief lymph drainage.

In a slightly later communication, Ramsay and Bennett (1943) studied the thyroid lymphatics in more detail by subcapsular injection of diluted Indian ink in anaesthetised animals. When the

injection was made into one lobe, the ink spread immediately through the lymph sac of that lobe and then across to the other lobe by a "bold" channel which marked the region of the isthmus, whether a parenchymatous isthmus was present or not. Smaller, additional isthmic vessels were also usually present. Two main groups of lymphatics drained the thyroid. The superior or cranial group, consisting of two to nine vessels, arose at the cranial pole of each lateral lobe and drained to a large, deep, cervical lymph node located dorsolaterally to each lobe. The inferior or caudal group arose at the caudal pole of each lateral lobe and drained caudally or caudolaterally into the tracheal nodes on the lateral or ventral trachea, to small nodes located in the superior mediastinum and to jugular nodes of varying size close to the jugular-subclavian venous junction.

In 18 of 32 animals, it was possible to identify thyroid lymphatics passing directly to veins without first passing through lymph nodes. These emptied either at the junctions of the internal or external jugular or the subclavian and jugular veins. In addition to these lymphatics, in many cases, other lymph vessels passed laterally or caudolaterally to empty directly into the cervical lymphatic trunks on either the right or left sides and in two animals into both. These "direct" vessels, which usually arose at the caudal poles of the glands whether they went to veins or to the cervical lymphatic trunks, were never the only draining vessels but were supported by the more usual type.

Schummer et al. (1981c) gave a detailed account of the lymphatic drainage of the cat's thyroid which differed to some extent from the above both factually and in terminology. They stated that the

thyroid was drained by two of the three groups of the deep lymph vessels of the neck (the lymphocentrum cervicale profundum), namely the cervicales profundi medii and caudales. The middle group is small and may be absent altogether whereas the caudal group is constant and particularly large.

The cervicales profundus medius is a small, round node of 1mm to 4.5mm diameter which is present in only one in four cats and then only unilaterally. It is situated against the cervical trachea and the internal jugular vein approximately halfway along the neck. It drains the thyroid and the cervical trachea and oesophagus, together with secondary lymph from the medial retropharyngeal node, its efferent route being to the jugular trunk. The cervicales profundi caudales (presumably corresponding to Ramsay and Bennett's inferior group) consists of one to six (usually two to four) round to elliptical nodes, 1mm to 3mm in diameter, located on the ventral surface of the trachea near the thoracic inlet at the bifurcation of the brachiocephalic veins. It drains the thyroid, the trachea and the oesophagus, with secondary lymph from nodes of the mediastinal, bronchial and ventral thoracic lymphocentres and from the middle deep cervical node. Secondary branches of the terminal part of the jugular trunk also enter these nodes. The efferent route is to the point of confluence of the external and internal jugular veins (the "venous angle") or, if only one of these veins is present, to its junction with the ipsilateral subclavian vein.

In a paper describing investigations on cats, rabbits and sheep, Daniel, Excell, Gale and Pratt (1962) stated simply that ten to 15 lymphatic trunks carried away thyroid lymph. In a further contribution, dealing with cats, rabbits, rats and monkeys, Daniel,

Gale and Pratt (1963) remarked that the thyroid was richly supplied with lymphatics which lay closely adjacent to the parenchymal cells. In both papers, these statements referred generally to the species mentioned.

Innervation

While detailed descriptions of the innervation of the thyroparathyroid apparatus of man date from the early part of this century (e.g. Rhinehart, 1912), specific information on the subject in cats is sparse. Nicholas and Swingle (1924a), while admitting they had made no special study of the subject, reported that the nerve supply to the feline parathyroids consisted entirely of sympathetic fibres from the perivascular plexuses. Soderberg (1958) stated that the main innervation of the cat and rabbit thyroids consisted of relatively large fibres from the vagus running chiefly in the superior and the recurrent laryngeal nerves. The recurrent nerve followed the caudal thyroid vein on each side of the trachea. Fibres from the cervical portion of the sympathetic trunk followed the cranial thyroid arteries but also joined both the superior and the recurrent laryngeal nerves.

McDonald (1975, 1980), discussing vertebrates in general, similarly reported that the thyroid had a rich supply of sympathetic nerves which entered the gland in association with the blood vessels. These nerves were thought to regulate only the blood supply to the organ, since transplantation did not affect thyroid function.

Schaer (1981) stated that the principle nerve supply to the feline thyroid was derived from the cranial cervical ganglion and the cranial laryngeal nerve. Sympathetic nerve innervation also

came from the cranial cervical ganglion. Thyroidal sympathetic adrenergic nerve terminals around vessels and follicles were rare.

3.4 ACCESSORY THYROID AND PARATHYROID TISSUE

Although Huguenin (1926) reported that he was unable to find "ectopic" (meaning accessory) thyroid tissue in 50 cats which he examined in detail, it is generally accepted that such tissue is not uncommon in practically any position between the tongue and the heart (Nicholas and Swingle, 1924a; Winiwarter, 1935; Schaer, 1981; Noxon, Thornburg, Dillender and Jones, 1983). In man, it results from the dislodging of primordial thyroid cells during the embryonic migration of the thyroid (Arey, 1966; Rogers, 1971) and it is believed that the situation is probably similar in the cat although experimental data have not been reported (Olsen, 1982; Noxon et al., 1983). The latter drew attention to the difference between ectopic and accessory thyroid tissue. Ectopic tissue results from premature or late separation of normal thyroid tissue from the embryonic heart as it migrates caudally. In this situation, the corresponding gland is missing from its normal position. Despite the variations in terminology, ("ectopic", "secondary" or "accessory") the descriptions here refer to true accessory tissue.

Vincent and Jolly (1906) sectioned the neck and mediastinal tissue of seven cats and found an accessory thyroid in one animal close to one of the thyroid lobes. Tehver (1940) described the presence of a poorly encapsulated "secondary" thyroid measuring 3mm by 1.5mm by 1.5mm within the tongue muscle of a cat.

Nicholas and Swingle (1924a) defined accessory parathyroid glands as "aggregations of parathyroid tissue other than the usual four glands, which have no intimate anatomical relation to the thyroid lobes". They found that what they described as "supernumerary" glands occurred fairly frequently in close proximity to the usual four parathyroids. However, their work involved the study of surgically-induced feline hypoparathyroidism and because these supernumerary glands were removed routinely during thyroidectomy, they did not consider them to be truly "accessory".

Despite Vincent and Jolly's report (1906) that they were unable to find accessory parathyroid tissue in the neck and mediastinal tissue of any of seven cats examined in detail, other authors almost unanimously agree on its existence, although its derivation is disputed. Harvier and Morel (1909) felt that it resulted from the origin of the third and fourth branchial clefts and their intimate developmental association with the thymus anlage, together with the shifting and migration of tissue which occurs in the cervical region during embryological development. Winiwarter (1935), however, believed that it did not result from parathyroid fragmentation but from tissue mutation of the thyroid and thymus. He found that although such tissue was invisible macroscopically, it could usually be detected microscopically between the thymus and the thyroid, in the larynx or towards the base of the heart. The number of accessory glands varied between five and eight.

There is considerable disagreement about the frequency with which accessory parathyroid tissue occurs in cats. Harvier and Morel (1909) identified it in the cervical thymus of 50 per cent of "less than five cats". Farner and Klinger (1920) believed that

such tissue was practically universally present in cats because 20 per cent of animals which they experimentally parathyroidectomised failed to show evidence of tetany. Shapiro and Jaffe (1923) reported that 12 per cent of cats which they examined had accessory parathyroids in their thoracic thymus. Nicholas and Swingle (1924a and b) disputed Harvier and Morel's figures and pointed to the small number of cats they had examined, but reported accessory parathyroids in 35 per cent of 107 animals.

3.5 HISTOLOGY

Although there are numerous accounts of the microscopic anatomy of the thyroid gland of the domestic animals generally, there are few specific descriptions of feline thyroid histology. The following account is taken from general veterinary histology works with specific details about the cat being added where such information is available.

The thyroid capsule (referred to previously) gives off septa or trabeculae of varying thickness to the interior of the gland. These are continuous with sparse, loose, interstitial connective tissue which divides the lobes into interconnected lobules (Trautman and Fiebiger, 1952). The feline thyroid has a relatively homogeneous structure, consisting of numerous follicles (also termed auri or vesicles) ranging from 10u to 15u interior diameter. Large follicles (up to 100u diameter) are rare but occur more frequently on the periphery. The central and peripheral zones are relatively indistinct from one another (Radacot, 1957). In young animals, the follicles often retain their embryonic continuity and are usually

smaller than in adults (Trautman and Fiebiger, 1952). The follicles are usually filled with colloid which may appear homogeneous or finely granular, and is usually acidophilic although it may show degrees of basophilia. It stains red with azocarmine, blue with aniline blue and is periodic acid-Schiff (PAS) positive due to its content of glycoprotein (Dellman, 1971; Dellman and Brown, 1981).

The follicles are completely closed and are surrounded by a network of delicate reticular fibres which are continuous with the capsular trabeculae (Trautman and Fiebiger, 1952). They are lined with a single layer of cells with a secretory polarity directed towards the follicular lumen. In the resting state, the cells are low cuboidal or even squamous. In the cat they average between 8u and 12u in height and have dense colloid (Radacot, 1957). When stimulated, the cells become cuboidal or columnar and the colloid is dissolved. The cells are joined to each other by terminal bars (Trautman and Fiebiger, 1952). The epithelium is surrounded by a basement membrane, sparse connective tissue, a dense network of capillaries possessing fenestrated endothelium similar to that in other endocrine glands, and lymph vessels (Dellman, 1971; Dellman and Brown, 1981).

The number and extent of the various cytoplasmic organelles vary according to the degree of activity of the cells. The round nucleus is in the basal portion of the cell and is surrounded by rough-surfaced endoplasmic reticulum. Mitochondria, ribosomes and polysomes are distributed throughout the cytoplasm. The Golgi apparatus lies between the nucleus and the apical cell surface which bears microvilli extending into the colloid. The apical cytoplasm also contains two types of vesicle. Apical vesicles originate from

the Golgi complex and contain thyroglobulin which is secreted by exocytosis into the follicular lumen. Large, membrane-bounded, colloid droplets arise by absorption into the follicular cells and merge with lysosomes, forming phagolysosomes in which thyroglobulin is broken down (Dellman and Brown, 1981; Schaer, 1981).

Parafollicular cells (light cells or C cells) are considered to be the source of calcitonin. In the cat, they are round or oval and lie singly or in small groups evenly distributed throughout the gland (Mietkiewski, Zabel and Linke, 1974). These authors found the reactions for alpha-glycerophosphate dehydrogenase and cholinesterase to be particularly good markers for these cells. They contain a well-developed Golgi apparatus and a large number of membrane-bounded granules and mitochondria (Dellman, 1971).

Tehver (1940) reported on the histological structure of the accessory thyroid tissue he identified in a cat's tongue. Only the larger follicles lying close to the periphery contained colloid but otherwise the tissue corresponded generally to that of normal thyroid gland.

Winiwarter (1935) reported that accessory parathyroid tissue was structurally identical to normal parathyroid tissue.

Both physiological and experimental situations may affect the morphology of the thyroid gland. Trautman and Fiebiger (1952), commenting generally about domestic animals, stated that thyroid histology changes with advancing age. The epithelium becomes flatter and there is an increase of fat and pigment granules in the cells. Pflugfelder (1955) found little change in thyroids of cats less than 16 years of age, but in a single 20-year-old animal, there was marked hyperplasia and formation of large follicles centrally,

although normal follicles were found at the periphery. Hurtrel (1974), in a quantitative study of the C cells of the left thyroid of 11 cats, identified a regression in cell density with advancing age but the small number of animals studied did not permit any general conclusions to be drawn.

Lowe (1930), in a study of the thyroid glands of four lactating cats, found no obvious differences between them and controls. Radacot (1957) examined the thyroid glands of 33 cats which were either pregnant (15 animals) or lactating (24 hours to six weeks post-partum, 12 animals) and compared them with six controls. He found little difference between the glands of non-pregnant and pregnant individuals. However, marked changes occurred at the beginning of lactation, with complete colloid resorption and the disappearance of large and medium sized follicles and their replacement with small follicles up to 5u internal diameter which were devoid of colloid. These were produced either by the regrouping of cells surrounding empty follicles or the proliferation of large acidophilic cells. The follicular epithelium was increased in height. Animals which had undergone parturition but not lactation showed thyroid histology similar to controls, confirming that the changes resulted from lactation and not simply from giving birth. After six weeks of lactation, there was a reaccumulation of colloid within the follicles which remained smaller than controls.

Sarrat (1966a and b) deprived cats of food and water and found their thyroids to be in a "regressive" state. He described large cells within the follicles and claimed that they were secretory and not simply desquamated. However, after subjecting 20 cats to hunger and thirst for 21 days, Vazquez (1974) claimed that the thyroids

were hyperactive in all animals. They consisted of many small follicles with barely perceptible lumina. The epithelium changed from cuboidal to columnar and, within the majority of cells, there were periodic acid Schiff (PAS) positive colloidal droplets, particularly at the apices.

3.6 THYROID PHYSIOLOGY

Introduction

There have been relatively few studies directed specifically at thyroid function in the cat. However, reactions leading to the formation of iodotyrosines and T₄ are well-established biochemical processes in animals; all vertebrates that metabolise iodine into an organic form produce T₄ and variable relative proportions of a triiodinated form of the thyronine molecule, and iodination of tyrosine molecules always takes place in a characteristic protein which in the mammalian thyroid is known as thyroglobulin (Gorbman, 1978). Thus, in the absence of information specifically to the contrary, feline thyroid physiology might reasonably be considered to be similar to that of man and the dog. The following account has been compiled from a large number of publications but, in particular, from those by Werner and Ingbar (1978) (discussing man) and Rosychuk (1982), Belshaw (1983) and Ferguson (1984) (discussing the dog), with specific details on the cat being added where such are available.

The metabolically active thyroid hormones are the iodothyronines, L-thyroxine and L-triiodothyronine and both have been repeatedly identified in feline serum (reviewed by Thoday, Seth and Elton, 1984). T₄ is the main secretory product of the thyroid.

However, T₃, the most active thyroid hormone, as well as smaller amounts of rT₃, an inactive product, and subsequent de-iodination products 3,3¹-diiodothyronine (3,3¹-T₂) and 3¹,5¹-diiodothyronine (3¹,5¹-T₂) have been found in venous effluent from canine thyroids (Laurberg, 1978, 1980 a and b, 1981). The structural formulae of these compounds are shown in Fig. 3.1.

Hormone synthesis and secretion

The thyroid gland is unique among the endocrine glands in that an integral part of its hormones is a micronutrient, iodine, which is available to the animal in only limited amounts. The daily maintenance iodine requirement for adult cats varies depending on the nature of the food. On purified diets, thyroid status remains normal with a daily dietary intake of approximately 70ug (Scott, 1965). However, the high-protein diet needed by cats imposes a relatively high iodine requirement because there appears to be a synergistic relationship between the requirements of iodine and calcium and the calcium content of meat is low (Roberts and Scott, 1961).

On high-protein diets, the daily iodine requirement for adult cats has been variably quoted as 150ug (Scott, 1965), 150 to 400ug (Scott, 1966, 1975a), 100ug/kg (Kallfelz, 1975) and 200 to 400ug (P.P. Scott, 1984). There are additional requirements during lactation as iodine passes into the milk in considerable quantities and additional T₄ is necessary for milk production. Fish and fish products, including cod-liver oil, are excellent sources of iodine: cats on a fish diet take in 400 to 500ug iodine daily. Experimentally, 5mg to 10mg potassium iodide daily are tolerated by normal cats which excrete the excess in urine (Scott, 1965, 1966,

1975a). Daily iodine requirements for growing kittens have been reported as 100 to 150 μ g on a high-protein diet (Greaves, Scott and Scott, 1959) and 0.01 to 0.02mg on a dry diet (Scott, 1965).

Knigge (1961) measured the stable iodine (^{127}I) concentration of the thyroids of 40 cats by an unspecified method. The cats were fed on the same commercial (unspecified) cat food. There was considerable variation, the mean \pm sd being $67.5 \pm 9.6\mu\text{g}$ per 100mg.

Most iodine in the diet is reduced to iodide in the gastrointestinal tract and absorption of iodide begins immediately (Belshaw, Cooper and Becker, 1975).

Inorganic iodide enters the thyroid follicular cells and is transformed through a series of metabolic steps into T4 and T3. Hormone synthesis involves (i) active transport of iodide, (ii) iodination of tyrosyl residues of thyroglobulin, (iii) coupling of iodotyrosine residues within thyroglobulin to form T4 and T3, (iv) proteolysis of thyroglobulin with release of free iodotyrosines and iodothyronines and secretion of iodothyronines into the blood, and (v) reutilisation of the liberated iodide (Taurog, 1978).

(i) Active transport of iodide. Iodide is concentrated from the extracellular fluid against a concentration gradient by a mechanism variably referred to as the iodide transport mechanism, the iodide concentrating mechanism, the iodide pump or the iodide trap, by an active transport system in the basal membrane of the thyroid follicular cell (Andros and Wollman, 1967). Cellular integrity is required for this transport (Wolff, 1964). Once inside the cell, iodide diffuses down a concentration gradient to reach the apical membrane and thus into the follicular lumen (Werner, 1982).

Gastric mucosa, salivary glands, mammary glands, choroid plexus, placenta and the skin also concentrate iodide in man (Taurog, 1978).

(ii) Iodination of tyrosyl residues of thyroglobulin.

Thyroglobulin, a large, iodinated glycoprotein with a molecular weight of 670,000 and a sedimentation coefficient of 19S, is produced within the thyroid cell. After synthesis on the rough endoplasmic reticulum, non-iodinated thyroglobulin migrates to the Golgi apparatus and, during this time, glycosylation occurs. Cytoplasmic vesicles containing thyroglobulin fuse with the apical membrane and are released by exocytosis into the follicular lumen. Iodide present there is inserted in the 3, or 3,5 positions of tyrosine residues on the thyroglobulin, forming moniodotyrosine (MIT) and diiodotyrosine (DIT), processes catalysed by a peroxidase (Ferguson, 1984).

(iii) Coupling of iodotyrosine residues within thyroglobulin to form T4 or T3. Two DIT residues couple via an ether linkage to form T4; one molecule of MIT and one of DIT couple to form T3.

(iv) Proteolysis of thyroglobulin with release of iodotyrosines and iodothyronines and secretion of iodothyronines into the blood. Secretion begins by the ingestion of thyroglobulin into the follicular cell in the form of colloid droplets. Ingestion occurs either by micropinocytosis (possibly the most important mechanism in basal secretion) or by pseudopod formation from the surface of the follicular cell (seen particularly after pharmacologic doses of TSH but not in resting glands). The colloid droplets acquire proteolytic enzymes from lysosomes, probably at the time of formation (Nunez, Belshaw and Gershon, 1972) and then migrate to the base of the cell. The iodothyronines

diffuse from the lysosomes and then from the cell into the extracellular space, from which they enter the adjacent capillary.

Both Ramsay (1938) and Ramsay and Bennett (1943) drew attention to the extensive lymphatic spaces of the cat's thyroid and suggested that they played an important role in the passage of secretion from the gland. Evidence consistent with this was presented by Daniel et al., (1962). Two to four days after injecting ^{131}I subcutaneously into cats, they collected thyroid gland lymph and thyroid venous blood before and after the administration via an unspecified route of an unspecified dose of TSH. They considered the count rate per unit weight of sample measured in a scintillation counter to be an indication of thyroid hormone concentration, which, it must be said, is both crude and inaccurate. Eighty-eight to 99 per cent of the radioactivity in the lymph was protein bound. They subsequently calculated the ratio (R) of count rate per unit weight of the thyroid lymph or thyroid venous blood relative to that in systemic blood. R was always considerably greater for thyroid lymph than for thyroid venous blood. After the administration of TSH, there was a rise in R in both calculated values. They concluded that the high count found in lymph must mean that a significant proportion of the thyroid hormone was carried away via lymphatics.

In a further experiment on ten cats, Daniel, Gale and Pratt, (1963) found that after TSH administration, R for thyroid lymph compared to thyroid venous plasma ranged from 25.8 to 59.3. Using the same technique, Daniel, Gale, Plaskett and Pratt (1963) chromatographed the thyroid lymph of cats one to two hours after giving TSH. Although they expected to identify mainly T₄, 70 to 90 per cent of the total radioactivity behaved like an iodoprotein

resembling thyroglobulin and failed to migrate. Most of the remainder was T4 and inorganic iodide. They eliminated experimentally the possibilities that this high concentration of thyroglobulin was due to manipulation of, or radiation damage to, the gland. This work throws some doubt on the group's initial conclusions about the importance of lymphatic drainage of secreted T4.

Taurog, Porter and Thio (1964) carried out a series of experiments in which they studied the compounds present in feline thyroid venous plasma after previous injection with ^{131}I . Forty minutes to two hours after the administration of 1iu or 1.4iu TSH, the T4 concentration increased approximately seven times but T3 or rT3 could not be demonstrated. In a further experiment, they injected two cats with 10uCi of ^{131}I daily for 13 days and then collected thyroid venous plasma before and after injection with 1iu TSH. No MIT and DIT could be demonstrated despite an increase in T4 of five times the basal value. In one cat which received 200uCi of ^{131}I , DIT was not present in the pre-TSH sample but was found after the administration of 1.4iu of TSH.

(v) De-iodination of iodotyrosines within the thyroid and conservation of the liberated iodide. Uncoupled MIT and DIT in thyroglobulin are also released during proteolysis of colloid droplets. In man, they are efficiently de-iodinated within the follicular cell and most of the iodide generated is re-used. In the dog, most of the iodotyrosine is released back into the circulation (Belshaw, 1983).

Taurog, Porter and Thio (1964) administered 10uCi of ^{131}I to cats for seven days and 2iu TSH 110 minutes before

thyroidectomy. Large MIT and DIT, T4 and iodine peaks were found in chromatographed thyroid digests. A small T3 peak was also identified but there were no unequivocal rT3 or T2 peaks. Because there was no demonstrable MIT and DIT in the circulation but high levels in the thyroids, the authors concluded that de-iodination of iodotyrosines in the feline thyroid must be very efficient.

Thyroid hormone transport and metabolism

All body T4 is initially secreted by the thyroid. In contrast, T3 production in the euthyroid state is variably a result of extrathyroidal conversion of T4 to T3 by de-iodination of its outer ring (5¹-de-iodination). In man, approximately 80 per cent of T3 is produced in this way (Braverman and Vagenakis, 1979), whereas in dogs the figure ranges from 37 to 60 per cent (Belshaw et al., 1974). Most tissues can convert T4 to T3 but the liver and kidney are the most active in this respect. T4 may also be de-iodinated in its inner ring (5-de-iodination) to produce the metabolically inactive rT3. In man, rT3 is almost entirely derived from extrathyroidal de-iodination of T4 (Chopra, 1978) with the corresponding figure in the dog being approximately 90 per cent (Laurberg, 1978). rT3 has not been described in the cat.

Thyroid hormones are carried in blood, bound to plasma proteins. In man, under normal physiological conditions, the majority of T4 is bound to an inter-alpha-globulin, thyroxine binding globulin (TBG), and to a lesser degree to a thyroxine binding prealbumin (TBPA) with a small proportion being bound to albumin. T3 is also predominantly bound to TBG and to a small extent to albumin but not to TBPA (Nicoloff, 1978). In the dog, T4 is bound to albumin and three globulin fractions and T3 to albumin

and one globulin (Refetoff, Robin and Fang, 1970). The overall binding affinity of plasma proteins for T4 is lower in dogs than in man due to the absence of a globulin comparable to TBG (Belshaw, 1983).

A small amount of data is available on feline T4 binding proteins. Robbins, Apter and Rall (1957) and Robbins and Rall (1960) made brief reference to T4 serum binding in an unspecified number of cats. After adding variable amounts (0.008 to 2ug per ml) ^{131}I -T4 to serum, they carried out conventional and reverse flow electrophoresis on paper in barbital, pH 8.6. This latter method was employed to overcome the artefacts arising from absorption by the paper of the albumin-bound T4 in the globulin region. The results by the conventional technique were not reported, but by reverse flow, the fastest of the major components (thought to be albumin) contained a large radioactive peak. There was a second, poorly separated peak immediately behind the first.

Farer, Robbins, Blumberg and Rall (1962) gave further details of this technique and discussed the results more fully. Using autoradiography or Geiger counting with subsequent staining of the proteins with bromphenol blue, the second peak was found to contain substantial quantities of radioactive T4. It corresponded in position to the alpha-globulin (and, therefore, TBG) region of human serum.

Tanabe, Ishii and Tamaki (1969) performed reverse flow cellulose acetate electrophoresis with 0.07M barbital buffer, pH 8.6, on an unspecified number of cat plasma samples, each containing 5ug per 100ml ^{131}I -T4. Protein zones were stained with Ponceau 3R and bound T4 was demonstrated by autoradiography or by cutting the gel and

counting in a scintillation counter. Most of the T4 appeared bound to albumin and postalbumin and no radioactivity was found in the alpha-globulin zone. When increments of 20 to 295ug T4 per 100ml of plasma were added, the radioactivity in the postalbumin band did not disappear or decrease. As TBG is characterised by saturability at relatively low T4 concentrations compared to other T4-binding proteins, the authors argued that the alpha-globulin band of Farer et al. (1962) was probably postalbumin and not comparable with human TBG.

Scherzinger, Guzy and Lorcher (1972) carried out conventional cellulose acetate electrophoresis with 0.04M sodium veronal buffer on an unspecified (between four and ten) number of cat sera containing 0.08ug per ml ^{131}I -T4. The resulting trace was stained with Ponceau S and the distribution of the radioactivity determined with a radioactivity scanner. T4 appeared bound to the anodal edge of the albumin band with "sliding" into the globulin region, and the authors speculated that there were two T4 binding fractions lying close together. There was no noticeable change in the percentage distribution of radioactivity after the addition of exogenous T4 and they suggested that feline TBG had only a low capacity with most T4 being bound to albumin at normal concentrations.

Bigler (1976a) used immunoelectrophoresis to identify the T4 binding proteins of four healthy cats. After incubating with 2.5ug ^{125}I -T4 per ml serum, agarose electrophoresis was carried out in 0.1M glycine acetate buffer, pH 8.6. Following immune precipitation with anti-cat serum, the proteins were stained with Ponceau S and subsequently autoradiographed for eight weeks. T4 binding to albumin was demonstrated but the authors failed to identify a T4 binding globulin.

Investigations into feline T3 serum protein binding have not been reported.

In man, the kinetic consequences of the interaction between T4 and binding proteins are dramatic. T4 is a small molecule with a molecular weight of only 700 and would be expected to diffuse rapidly into interstitial fluid. However, because it is avidly bound to plasma proteins, its diffusion through the capillary barrier to target organs and tissues is retarded.

Even from the early studies of compartmental kinetics in man, it became clear that the extrathyroidal T4 pool was not homogeneous but should be considered as having functionally separate compartments. From work in sheep (Irvine, 1969) and its subsequent application to man, two different kinetic compartments have been identified. The rapidly equilibrating compartment is made up of the liver and kidney and the slowly equilibrating compartment of muscle, skin, brain, fat and other tissues (Nicoloff, 1978). The differences between the two probably arise because of variations in the permeability of their capillary walls to T4.

Compartmental kinetics are not as well defined for T3 but are probably similar. However, because T3 is less firmly bound to carrier proteins, it equilibrates much faster than T4 in each compartment.

In man, approximately 50 per cent of body T4 and 15 per cent of body T3 is found in the interstitial fluid and plasma compartments. Approximately 55 per cent of intracellular T4 but only five per cent of intracellular T3 is present in the rapidly equilibrating tissues. Some 80 per cent of all extrathyroidal T3 but only 22 per cent of extrathyroidal T4 is located in the slowly equilibrating tissues (Nicoloff, 1978).

In the dog, about 40 per cent of the extrathyroidal T4 is in plasma and most of the remainder is taken up by the liver. The total plasma-equivalent space of distribution is 12 per cent. Because T3 is less avidly bound to plasma proteins, it enters peripheral cells more readily and reaches a total distribution volume equal to 65 per cent of bodyweight (Belshaw, 1983).

Most studies suggest that only free (unbound) hormone is **metabolically** active, the bound fraction acting as a reservoir in situations of rapid increase or decrease of hormone supply to tissues. In dogs, estimates of plasma free T4 (FT4) have varied from 0.103 to 0.189 per cent (Furth, Becker, Nunez and Reid, 1968), to 0.155 to 0.303 per cent (Refetoff, Robin and Fang, 1970) of the total plasma concentration. Thus, some 99.7 to 99.9 per cent of circulating T4 in dogs is bound to plasma proteins. Because of the presence of the high affinity binding protein TBG, the FT4 concentration of human plasma is approximately one third to one fifth of the dog i.e. 0.03 to 0.05 per cent of total plasma T4. However, the dog's total T4 concentration is proportionately lower making its FT4 concentration very similar to that of man.

The proportion of free hormone is a primary determinant of the rate of fractional turnover. The T4 plasma half-life, therefore, is considerably shorter in the dog than in man and has been variably reported as 10.3 hours (Furth et al., 1968) and 15.9 hours (Kallfelz and Erali, 1973), whereas in man it is approximately seven days (Nicoloff, 1978). The half-life of T3 has been estimated to be five to six hours in the dog (Fox and Nachreiner, 1981) and 24 hours in man (Nicoloff, 1978).

T4 and T3 may be sequentially de-iodinated to produce the simple thyronine nucleus (T0). The resultant metabolites are not thought to have metabolic activity but complete de-iodination preserves iodine. Fifteen per cent to 20 per cent of the total daily disposal of T4 and T3 in man is conjugated to soluble glucuronides and sulphates (Nicoloff, 1978) and a much smaller amount is degraded by oxidative deamination and cleavage of the ether linkage. The conjugates may be excreted in bile or in urine. Those passed into the gut are hydrolysed back to T4 and reabsorbed, this entero-hepatic circulation being of importance in the preservation of total body T4.

In the dog, approximately 45 per cent of T4 turnover is via de-iodination with 55 per cent being by faecal secretion (biliary clearance of T4 being very much higher in the dog than in man). Seventy per cent of T3 turnover is via subsequent de-iodination and 30 per cent is secreted in the faeces (Furth et al., 1968; Belshaw, Barandes, Becker and Berman, 1974). Less than 15 per cent of T4 is reabsorbed in the dog (Furth et al., 1968) compared with 70 per cent of T4 and 100 per cent of T3 in man.

Sites and mechanism of action of thyroid hormones

T4 has been described by many workers as a prohormone with its activity being dependent on its mono-de-iodination to T3. While this conversion is responsible for approximately 85 per cent of its activity, T4, like T3 does have intrinsic thyromimetic actions, although studies in man and the rat have established that T3 is three to five times more potent (Ferguson, 1984).

Specific nuclear T3 receptors were described by Oppenheimer, Koerner, Schwartz and Surks (1972) and mediate effects dependent on

protein synthesis, possibly as a result of augmented transcription of DNA or modulation of RNA. The responsiveness of a tissue to thyroid hormone is dependent to some extent on the numbers of nuclear receptors it possesses. Thyroid responsive tissues such as liver and kidney contain high numbers of receptors whereas thyroid non-responsive tissues such as spleen and testis possess few. Specific T₃ binding to plasma membranes and mitochondria has also been demonstrated. These effects are much more rapid than those evoked by binding to nuclear receptors. Effects of thyroid hormone on membrane-bound Na⁺K⁺ATPase, amino acid transport and mitochondrial oxygen consumption have been demonstrated minutes to hours after administration. Additionally, the numbers of beta-adrenergic receptors and/or the activity of plasma membrane adenylate cyclase may be increased by T₃ administration (Werner, 1982).

The cellular role of T₄ appears to be of major importance in the regulation of CNS and pituitary function (Larsen, Silva and Kaplan, 1981).

Control of thyroid function

The hypothalamic-pituitary-thyroid-extrathyroidal axis. Detailed information about this axis in the cat is unavailable. In man, peptidergic neurones in the thyrotropic area of the hypothalamus secrete the tripeptide amide, L-pyroglutamyl-L-histidyl-L-prolineamide, thyrotropin releasing hormone (TRH) into the hypophyseal portal system. TRH acts on the thyrotrope cells of the anterior pituitary by binding to specific receptors that activate an adenylate cyclase-cyclic adenosine monophosphate (c-AMP) system. The effect of TRH on TSH secretion is not dependent on the

synthesis of protein and is extremely rapid, measurable increases in plasma TSH concentrations occurring within one to two minutes in both man and animals (Reichlin, 1978).

The thyroid is under the direct control of TSH. This is a glycoprotein of molecular weight in the range of 28,000-30,000, consisting of two peptide chains. The alpha chain is common to TSH, luteinising hormone (LH) and follicle stimulating hormone (FSH) within a species, whereas the beta chain has a different amino acid sequence depending on the hormonal activity to be expressed (Pierce, 1971). TSH attaches to receptors on the cells of the thyroid follicles, activates adenylate cyclase and enhances c-AMP production. It is likely, although not yet proven in all instances, that c-AMP mediates the effects of TSH on follicular cell growth, iodination and thyroid hormone synthesis and thyroid hormone secretion (Van Herle, Vassart and Dumont, 1979). Within minutes of TSH administration, there is enhancement of iodide binding to protein, iodothyronine synthesis, thyroglobulin secretion into the follicular lumen and ingestion of colloid via pseudopod formation. Increased trapping of iodide follows after several hours (Belshaw, 1983). TSH also increases the secretion of the de-iodination products, suggesting that significant T4 de-iodination occurs during secretion.

Knigge and Joseph (1971) reported the concentrations of TSH in the pituitary glands of normal cats. However, they used a modification of a human TSH assay and as TSH is species specific, the validity of the values obtained is debatable.

Recently, Lothrop, Tamas and Fadok (1984) described increases in serum T4 after intravenous TRH administration in six cats,

indicating a functional pituitary-thyroid axis with endogenous production of TSH by the pituitary gland and subsequent thyroid stimulation.

Inhibition of thyroid hormone production is achieved via a negative feedback system whereby plasma FT4 and free T3 (FT3) act directly on the pituitary. This feedback appears to involve both the production of an inhibitory protein that blocks TSH release and a decrease in the number of TRH receptors on the pituitary thyrotrope cells (Hinkle, Perrone and Schonbrunn, 1981). Thus, these effects take several hours. The pituitary rapidly de-iodinates T4 to T3 and thyrotrope nuclear T3 receptor occupancy is inversely proportional to TSH secretion. Despite T3 being the active intracellular hormone for TSH suppression, T4 is the less variable source of pituitary nuclear T3, and T4 elevation produces a more prolonged suppression of TSH release (Morley, 1981; Werner, 1982). However, it must be possible for T4 to affect TSH independently of plasma T3, or else the pituitary could not increase TSH with resultant goitre in iodine deficiency when plasma T3 is maintained at the expense of T4 (Larsen, Silva and Kaplan, 1981).

Small doses of thyroid hormones suppress and large doses completely abolish the TRH-induced release of TSH.

Knigge and Joseph (1971), in experiments with normal cats and cats in which the basal hypothalamus had been de-afferented, concluded that an inhibitory pathway from the preoptic area to some region close to the median eminence was involved in the negative feedback action of T4. Joseph and Knigge (1972) concluded that there was a similar pathway in kittens.

The hypothalamic hormone, somatostatin, has an inhibitory action on TSH release in man and the rat but its role in tonic regulation is still unknown. Its inhibitory action appears to be additive to that of T4 and T3 (Reichlin, 1978).

Extrathyroidal thyroid hormone metabolism may also act as a regulator of thyroid function by de-iodination of T4 to either T3 or the metabolically inactive rT3. Recently, it has been suggested that extrathyroidal de-iodination may be autoregulated (Lum, Nicoloff and Spencer, 1983).

Other mechanisms affecting thyroid hormone production

Iodine intake. Thyroglobulin stored within thyroid follicles allows for continued hormone secretion during iodine deficiency. During such time, there may be preferential T3 secretion relative to T4 because of increased formation of MIT relative to DIT (Belshaw et al., 1974; Werner, 1982). Because T3 is more potent than T4, this mechanism serves to maintain euthyroidism despite iodine deficiency.

A gradual increase in iodide intake, with a consequent increase in thyroidal iodide levels, results in increased stores of thyroid hormones. Beyond a certain level, excess iodide administration has an antithyroid effect as iodide binding to thyroglobulin is progressively inhibited. This is the Wolff-Chaikoff effect (Wolff and Chaikoff, 1948). The mechanism of this effect is unknown but Raben (1949) demonstrated that it depended on a critical level of iodide being reached in the thyroid rather than the circulation. However, the inhibition of thyroid hormone synthesis is followed by an "escape" phenomenon due to autoregulation of thyroid iodide transport. These autoregulatory phenomena are protective mechanisms, preventing massive thyroid hormone release after an iodide overload.

Sherwin (1982) investigated the ability of foetal, neonatal and adult cat thyroid glands to respond to excess iodide in vitro. He concluded that the autoregulatory effect of iodide transport develops at a later gestational time than the action on TSH-stimulated c-AMP formation.

Inherent rhythms. A number of factors have been suggested as producing an inherent rhythm in feline thyroid activity. Lowe (1930) thought that there was an annual thyroid cycle which could be overridden by physiological influences such as gestation.

Knigge (1961) identified differences in the plasma protein-bound iodine (PBI) concentrations of 40 normal cats between winter and summer, but the results were not statistically significant.

Randall and Littschwager (1967), Randall and Parsons (1970) and Rogers and Randall (1972) assessed seasonal variation in feline thyroid function by measuring ^{131}I or ^{125}I retention and excretion after the administration of Na^{131}I or Na^{125}I . A three to four month rhythm was identified with maximal activity occurring in autumn and minimal activity in spring. Using the same technique, Randall (1970) found that the thyroid function of ten normal cats was significantly correlated with the percentage sunshine, maximal activity lagging approximately one month behind times of maximal sunshine. He concluded that the behaviour of clouds correlated with feline thyroid function. Rogers and Randall (1972) even correlated the position of the planet Mercury with the thyroid activity of cats, although they remarked on the uncertainty of the relevance of this observation!

Woods, Wayt and Baker (1966) examined the radioactivity of the thyroids of 12 adult cats after intravenous or subcutaneous ^{131}I administration. Peak radioactivity occurred in the morning (mean time 07.14 hours) with minimum activity in the evening (mean time 19.42 hours). The cycles persisted in animals in which the thyroids had been transplanted to the tail region and in others after division of the spinal cord in the lower cervical region. No cycles occurred in two decerebrate animals, indicating the role of the CNS in the rhythm.

Nelson, Wolfangel and Stara (1966) assessed radioactivity in "blood" samples from 12 cats taken morning and evening for four days after Na^{131}I had been administered intravenously. In all cases, morning samples had a much higher concentration of radioiodine than the previous afternoon's specimens, suggesting possible diurnal cycling. Hoenig and Ferguson (1983) collected blood samples at two-hourly intervals over a 24 hour period from six cats and failed to find any diurnal variation in serum T4 concentration as assessed by double antibody radioimmunoassay. However, as thyroid ^{131}I activity, blood ^{131}I activity and serum T4 concentration are not measurements of the same functions, these results are not necessarily contradictory.

Donne and Wildgoose (1984) collected blood samples from two Devon Rex cats every two hours between 08.00 hours and 18.00 hours, and measured total serum T4 and T3 by solid phase, magnetic antibody immunoassay. T4 concentration rose minimally from 08.00 hours until 10.00 hours and then remained constant. T3 concentrations rose gradually but steadily from 08.00 hours to 16.00 hours and then decreased. Because only two animals were used and samples were taken over a ten hour period little can be deduced from this study.

In man, there is a minor nyctohemeral rhythm in TSH secretion (Van Haelst, Van Cauter, Deguate and Golstein, 1972), with peak secretion between midnight and 04.00 hours and a fall after 06.00 to 08.00 hours but the physiological significance of this is unknown. It seems likely that the circadian rhythms of TSH and of cortisol and, by extension, of adrenocorticotrophic hormone (ACTH) are independently controlled by the CNS and are not reciprocally related (Van Cauter, Leclercq, Van Haelst and Golstein, 1974). No data have been published on feline plasma TSH concentrations, a consequence of the lack of suitable bioassay or radioimmunoassay methods for feline TSH.

Factors affecting extrathyroidal metabolism of thyroid hormones

Effects of disease and malnutrition. The euthyroid sick syndrome. In man, a number of conditions (diabetes mellitus, hepatic and renal conditions, other chronic disease states, surgery, starvation, malnutrition) are associated with reduced extrathyroidal T4 to T3 conversion and, except in chronic renal failure, increased plasma rT3. This decrease in circulating T3 is probably a mechanism to conserve protein during illness and has been termed the "low T3" or "euthyroid sick" syndrome. The specific mechanism is not clear but it is speculated that there is inhibition of the 5¹-de-iodinase enzyme which de-iodinates T4 to T3 and rT3 to 3, 3¹-T2. This results in reduced T3 production and decreased rT3 clearance (Ferguson, 1984).

The total serum T4 concentration may also be reduced in the euthyroid sick syndrome and is termed specifically the "low T4 state of medical illness". It is usually associated with acute, severe conditions (Slag, Morley, Elson, Crowson, Nuttal and Shafer, 1981;

Kaplan, Larsen, Crantz, Dzau, Rossing and Haddow, 1982). Suggested mechanisms for this change include inhibition of plasma T4 binding, possibly by blocking of binding sites by immunoglobulin M (IgM) or immunoglobulin G (IgG) immune complexes (Chopra, Chua Teco, Nguyen and Solomon, 1979), decreases in plasma binding protein concentrations (Woeber and Maddux, 1981) and alterations in tissue T4 binding (Kaptein, Robinson, Grieb and Nicoloff, 1982). The free T4 concentration remains the same and patients are euthyroid.

The euthyroid sick syndrome is also believed to occur in the dog. However, in chronic renal disease, dogs have elevated plasma rT3. In a group of dogs with a variety of medical conditions in an intensive care unit, all had sub-normal T4 concentrations (Ferguson, 1984). The euthyroid sick syndrome has not been investigated in the cat.

Effect of drugs. A number of drugs have been shown to alter plasma thyroid hormone concentrations, either by changes in peripheral metabolism or in plasma or cellular binding (Kaplan, 1981; Wenzel, 1981). In dogs, plasma T4 concentration may be moderately to severely depressed by, for example, diphenylhydantoin, phenobarbital, phenylbutazone, o,p¹DDD and glucocorticoids (Belshaw, 1983). Plasma T3 concentration is lowered by prednisone (Woltz, Thompson, Kemppainen, Munnell and Lorenz, 1983). In man, diphenylhydantoin interferes with the binding of T4 to plasma proteins and increases T4 to T3 conversion. Phenobarbital increases biliary excretion of T4 and possibly T4 de-iodination. Phenylbutazone and o,p¹DDD interfere with T4 binding to plasma proteins. Glucocorticoids, both exogenous and when produced in excess endogenously, decrease the release of TRH, the TRH-induced

release of TSH and the T4 binding capacity of TBG. Dogs maintained for long periods on these drugs do not develop signs of hypothyroidism, euthyroidism probably being maintained by adequate levels of free T4 and T3. There have been no detailed studies of the exact mechanism by which each of these drugs affects total plasma T4 concentration in the dog.

In man, androgens decrease the concentration of thyroid hormone binding proteins and oestrogens increase them (Wenzel, 1981). Similar studies have not been carried out in dogs but pregnant bitches have been reported to have higher protein-bound ^{125}I (mostly T4) concentrations (Monty, Wilson and Stone, 1979). However, dogs with hyperoestrogenism have normal T4 and T3 concentrations but depressed responses to TSH (Gosselin, Capen, Martin and Targowski, 1980).

Effect of obesity. Overeating results in increased T3 production in man (Danforth, 1981) but the TSH response to TRH is not blunted and such people are euthyroid (Utiger, 1982). The total serum T3 and T4 concentrations of obese dogs are elevated (Gosselin et al., 1980). Similar investigations have not been carried out in the cat.

3.7 METHODS USED TO ASSESS NORMAL FELINE THYROID FUNCTION - MEASUREMENT OF CIRCULATING HORMONES UNDER BASAL CONDITIONS

Introduction

Despite the cat's popularity both as a pet and as a laboratory animal, its thyroid function has been little studied by comparison with that of the dog. This may have resulted, in part, from an apparent lack of thyroid-related disease in the former. The

following account reviews those tests which have been used to assess the thyroid function of healthy cats.

Protein-bound iodine (PBI)

Method principle. This test was one of the first in vitro techniques used to study human and animal thyroid function (e.g. Trevorrow, 1939; Riggs and Man, 1940). It measures chemically the amount of iodine that is co-precipitable with serum proteins. While no figures are available for the cat, at least 99.8 per cent of canine and 99.95 per cent of human T4 is bound to plasma proteins and 65 per cent of the T4 molecule is iodine (Lorenz and Cornelius, 1976). Thus, the PBI is a reflection of the serum T4 concentration, but will also reflect changes in the thyroid hormone transport proteins. Other non-hormonal iodoproteins that may be present will also be included.

In man, the diagnostic efficiency of the PBI was originally reported as being satisfactory for both hypothyroidism (e.g. Salter, 1947) and hyperthyroidism (e.g. Starr, Petit, Chancy, Rollman, Aiken, Jamieson and Kling, 1950). A number of early workers (e.g. Meier and Clark, 1958; Wilson, Dickson and Frost, 1961) also considered that the technique was suitable for measuring canine thyroid function. However, the inorganic iodine content of human serum is usually negligible (de Mowbray and Tickner, 1952), whereas canine serum contains high concentrations of inorganic iodides which may falsely elevate the PBI (Lorenz and Cornelius, 1976). Canine serum may also contain variable amounts of non-hormonal iodoproteins (Siegel, 1977). Thus it rapidly became clear that PBI was inadequate for diagnostic purposes in the dog.

Other sources of error in the use of the technique in both human and veterinary medicine include contamination by iodine in glassware (de Mowbray and Tickner, 1952), iodine administration to the patient in the form of drugs such as the anthelmintic DNP (Thompson and Michaelson, 1967), expectorants (Lorenz and Cornelius, 1976), iodised vitamins (Kaneko, 1980a), iodine containing radiographic contrast media (reviewed by de Mowbray and Tickner, 1952 and Kaneko, 1980a), and topically applied, iodine-containing antiseptics (Lorenz and Cornelius, 1976). Any drug which affects the concentrations of thyroid hormone binding proteins will also alter the PBI concentration (reviewed by Kaneko, 1980a). The mercury content of some diuretics can cause an artificial decrease in the PBI level by interfering with the chemical estimation of PBI (Siegel, 1977).

Because of these problems and the advent of more convenient and specific radioimmunoassay methods, the PBI is now obsolete in human medicine (Toft, Campbell and Seth, 1981) and is no longer considered a reliable test of thyroid function in dogs (Siegel, 1977).

Clinical studies. There are no critical reviews of the value of PBI concentrations in the assessment of feline thyroid function other than reports consisting simply of statements of ranges in veterinary texts or in medical research papers. These are summarised in Table 3.1.

Although the higher values reported in the earlier studies do not appear to have been confirmed, there is reasonable agreement that the mean PBI in normal cats is approximately 230nmol/l (which is equivalent to 57nmol/l T₄).

In vitro radiolabelled triiodothyronine uptake tests

Method principle. As previously discussed, thyroid hormones are carried in blood, bound to plasma proteins. In man, TBG has a high affinity for, and binds the majority of, T₄. T₃ is also predominantly bound to TBG but less avidly than T₄ which it cannot displace from binding sites on TBG (Luick, 1971). Thus, for a constant TBG concentration, the thyroid hormone concentration is inversely proportional to the number of unoccupied binding sites. The erythrocyte appears to be a secondary binding site for circulating thyroid hormones. Using ¹³¹I labelled T₄ and T₃, Crispell, Kahana and Hyer (1956) showed in vitro that the uptake by human red blood cells (RBCs) was consistently greater for T₃ than for T₄ and that the presence of plasma markedly decreased T₄, and to a lesser extent, T₃ erythrocyte binding. As thyroid hormone concentrations rise, the degree of saturation of the plasma protein binding sites by T₄ decreases their availability for T₃ and the surplus is bound to the red cell surface. This is the basis of the erythrocyte or red cell T₃ uptake test (RBC T₃ uptake test).

This test was devised by Hamolsky, Stein and Freedberg (1957) who estimated thyroid function based on the in vitro uptake by the erythrocytes of ¹³¹I-T₃ added to a sample of human whole blood. After separating and washing the RBCs, they counted their radioactivity and expressed it as a percentage of the total blood radioactivity, corrected to a haematocrit reading of 100. In 231 determinations on 209 euthyroid subjects, the values for the corrected erythrocyte uptakes ranged from 10.3 per cent to 17 per cent with a mean of 13.9 per cent, levels rising in hyperthyroidism and falling in hypothyroidism.

In 1961, Sterling and Tabachnik reported a modification of the technique using the patient's serum instead of whole blood and an anion exchange resin in place of erythrocytes (T3 uptake test). The resin's avidity for ^{131}I -T3 was less than TBG so the test worked in the same way as the RBC T3 uptake test. The advantages of the method were that it excluded complications due to haemolysis and did not require the use of a haematocrit correction factor. Thereafter, a number of other absorbents were used, e.g. incorporating the resin into a sponge or using charcoal, haemoglobin saturated charcoal, silicate or Sephadex and a number of kits were produced commercially. The normal range for the resin sponge uptake in man is 23.2 per cent to 32.6 per cent uptake (Goolden, Gartside and Orsorio, 1965).

The T3 uptake tests found widespread use as thyroid function tests in man because of their simplicity and convenience. Non-thyroidal organic iodides did not interfere with, or caused only a slight increase in, uptake (Hamolsky, Stein and Freedberg, 1957). Currently, they are also useful during pregnancy and as an adjunct to other tests (see later). However, a necessary prerequisite for the diagnostic use of the test is a high affinity thyroid hormone plasma binding protein. There is considerable debate as to whether such a protein occurs in cat plasma.

Clinical studies. The results from these are summarised in Table 3.2.

Wilson, Dickson and Frost (1961) are the only workers to have used the RBC T3 uptake test to assess feline thyroid function. Unfortunately, animals studied were clinical patients in the authors' veterinary hospital or from experimental projects at their

College of Veterinary Medicine and so could not be classed as "healthy". They used the same procedure as Hamolsky, Stein and Freedberg (1957), but corrected to a standard packed cell volume (PCV) of 40. The mean value for the cat was the highest of all the species studied (horse, cow, pig, sheep, dog).

Tanabe, Ishii and Tamaki (1969) measured the ^{131}I -T3 uptake on acetalised polyvinyl alcohol sponges in a number of species by a method originally described by Inagaki (1965). Only one cat was tested but its uptake was the highest of all the mammalian species studied (man, cow, sheep, goat, rabbit, guinea-pig and mouse) with the exception of the latter.

Kallfelz and Erali (1973) used a commercially available resin sponge uptake test (Triosorb-125, Abbott Radiopharmaceuticals) to assess thyroid function in 14 cats of varying ages. No details of technique were given. The mean uptake of 57.2 per cent was more than ten per cent higher than any other species studied (dog, pig, cow, goat, sheep and horse) and compared with a value for man of approximately 30 per cent. They concluded that the concentration of T4 binding proteins or their affinity for T4 must be different in animals from man.

Ling, Lowenstine and Kaneko (1974) evaluated a commercial kit method of T3 uptake using silicate particle adsorption (Tri-Tab, Nuclear Medical Laboratories) in cats presented for sterilisation. The mean uptake in 39 cats was almost identical to that found by Kallfelz and Erali (1973).

Lorenz and Cornelius (1976) reported the T3 resin sponge uptake values of an unspecified number of cats by an unspecified method. Their normal range was identical to that given by Ling, Lowenstine

and Kaneko (1974). However, the last named authors described their technique as silicate particle adsorption.

With the advent of commercially available, highly specific antibodies against T₃, Anderson and Brown (1979) reported T₃ uptake values utilising them as the uptake medium. In their technique, serum and buffer containing ¹²⁵I-T₃ were incubated in plastic tubes coated with a T₃ antibody (Immunotubes, Smith Kline). Competition for the ¹²⁵I-T₃ was between the antibody and the unsaturated binding sites of what the authors called "TBG" in the serum. The bound and free ¹²⁵I-T₃ were separated by decanting. Ninety-two healthy cats of several breeds and a wide age range were studied.

Because neither naturally-occurring hypo or hyperthyroidism had been documented in cats at the time of these studies, it was not possible for these workers to make any comment about the specific value of these tests in assessing feline thyroid function. However, both Anderson and Dorner (1971) and Kallfelz (1973), in work with thyroidectomised dogs, documented the insensitivity of the T₃ uptake tests in detecting changes in canine thyroid gland function. They commented that this resulted from differences in binding affinity between the plasma proteins of the dog and man, with the consequence that the test did not alter significantly even with marked changes in thyroid activity. Such differences could also be a consequence of smaller concentrations of binding proteins with the same binding affinity. Neither Scherzinger, Guzy and Lorcher (1972) nor Bigler (1976a) were able to identify the presence of a high-affinity thyroxine binding alpha-globulin in cat serum, although the first named authors felt that this might be due to inadequate separation techniques being employed. Both reports stated that the T₃ uptake

test was consequently not suitable for assessing thyroid function in cats.

In general, the T3 resin uptake and Immuntube techniques in cats show satisfactory agreement but give different results from the initial method using RBCs. T3 uptake tests have played a diminishing role as a test of thyroid function in man, and their use is now essentially limited to the estimation of the concentration of unoccupied TBG sites for the purpose of calculating the "free thyroxine index".

Competitive protein binding (CPB) methods

Method principle. This approach provided the first direct measurement of thyroid hormone concentrations in the cat. The procedure is similar to the radioimmunoassay (RIA) methods that were introduced later but differs in the important respect that TBG is used as the binding reagent rather than an anti-T4 antibody.

The procedure involves the extraction of T4 from patient serum and its subsequent incubation with a fixed amount of human TBG in the presence of ^{125}I -T4. The ^{125}I -T4 is displaced from the TBG by the patient T4 in an amount proportional to the amount of patient T4. The displaced ^{125}I -T4 is then removed from solution by an anion exchange resin or by silicate particles. The ^{125}I -T4 on the adsorbent or, alternatively remaining in solution on the TBG, may then be counted and from standards treated in the same way, the total serum T4 of the patient can be interpolated from the standard curve.

This test was first described by Ekins (1960) and later was developed and simplified by Murphy (1964) and Murphy and Pattee (1964) using ^{131}I -T4 and Sephadex columns. Murphy and Jachan (1965)

further modified the method by using ion-exchange resin and dispensing with the Sephadex columns. Subsequently, the test was modified by others and became commercially available in a number of kit forms.

The T4 CPB test is a direct indicator of T4 concentration. Unlike the PBI, it is not affected by iodides (Kaneko, 1970, 1980a) and the most commonly-occurring sources of interference are medications which alter the TBG concentration (e.g. oestrogens) or compounds which compete with T4 for binding sites on the binding proteins. Only diphenylhydantoin and salicylates compete when given in large amounts, but in small doses even this interference is negligible (Sparagana, Phillips and Kucera, 1969). These studies indicated that T4 CPB was a major improvement over earlier methods of thyroid function testing.

Clinical studies. Table 3.3 summarises data on CPB measurements of T4 in healthy cats.

Scherzinger, Guzy and Lorcher (1972) were the first people to use the test in the cat. They estimated the T4 concentration of a sample of pooled normal cat serum, using ^{125}I -T4, human TBG and an anion exchange resin as described by Murphy and Jachan (1965). They reported a value which was in good agreement with later results from Scherzinger and Grosser (1972) who used the same method on samples from five cats.

Kallfelz and Erali (1973) evaluated a commercially available kit designed for use in man (Tetrasorb-125, Abbott Radiopharmaceuticals). This technique initially involved alcoholic extraction of T4 from the test serum. As not all of the T4 was extractable in this way, recovery of T4 on extraction had to be

separately estimated in each experimental run. The radioactive label was ^{125}I -T4 which was added bound to TBG, with the adsorption medium being a resin sponge. These authors' values appeared somewhat lower than those recorded in previous studies, but similar results were later recorded by Scott (1975b), using the same kit.

Bigler (1976b) also used the Tetrasorb-125 kit in measuring the total serum T4 concentration of 110 healthy cats of both sexes and varying ages. Unmodified, the kit gives optimum results between 51.5nmol/l and 205.9nmol/l. Because of the low concentrations of T4 found in cat serum, he modified the technique by using three times the volume of alcohol-extracted T4. His results were lower than those previously reported by Kallfelz and Erali (1973) using the same method without modifications.

Ling, Lowenstine and Kaneko (1974) used an alternative commercial method of T4-CPB (Tetra-Tab, Nuclear-Medical Laboratories) with cat serum. This technique employs ^{125}I -T4, human TBG and inorganic silicate particles as the adsorbent. The mean T4 concentration of 40 healthy cats of a number of breeds, both sexes and varying ages was 12.3nmol/l with a range of 1.3nmol/l to 32.2nmol/l. While they included two additional standards of 19.3nmol/l and 38.6nmol/l to allow for the low T4 concentrations found in cat serum, the difficulties in quantitating low T4 concentrations in an assay designed for the higher normal values in humans raise some doubts concerning the reliability of the lower limit of their reference range.

Kaneko (1980a) also used CPB to measure T4 ranges in the cat but did not specify the particular method.

Overall, the data in Table 3.3 show that there is no clear agreement on normal T4 concentrations in the cat as measured by CPB, although the initial estimates appear, in retrospect, to have been too high. The best estimate of the normal mean appears to be in the range 10nmol/l to 30nmol/l.

Most workers consider T4 CPB to be less specific than T4 determinations by radioimmunoassay (T4 RIA) because, in addition to the total T4, CPB measures approximately 35 per cent of the total serum T3 (Scherzinger, Guzy and Lorcher, 1972). However, Kaneko, Baker and Mills (1975) found no difference in statistical comparisons of T4 CPB with T4 RIA. This may have resulted from low values due to under-recovery on extraction, being compensated for by some degree of non-specificity in the assay.

Radioimmunoassay of thyroxine and triiodothyronine

Method principle. The principle of RIA was first described by Yalow and Berson (1960) in their now classic paper on the measurement of insulin using specific insulin antibodies. The method offers a unique combination of specificity, sensitivity, precision and practicality and its application to the measurement of other hormones has revolutionised endocrinology.

There are three requirements for successful RIA: (i) an antibody which has high affinity and specificity for the analyte (or antigen) to be measured, (ii) a radiolabelled form of the analyte, and (iii) a suitable method for separating in solution, the antibody-bound and "free" forms of the labelled analyte.

The analyte reacts with the specific antibody, which is present at low but constant concentration, in the presence of radiolabelled analyte. The proportion of the total analyte which is

antibody-bound decreases as the amount of added analyte increases. The labelled analyte (usually, though not necessarily, the same as the substance being measured) is distributed between the antibody-bound and free forms in the same way as the non-labelled analyte. This partitioning of labelled analyte can be measured using one of a variety of methods that exploit differences in molecular size or charge, adsorption or immunological or solubility properties between antibody-bound and free moieties. Following this separation, the radioactivity in the antibody-bound or free fraction is measured and by comparison with an appropriate standard curve, the concentration of the test antigen can be interpolated.

The extension of the RIA technique from protein analytes to include low molecular weight analytes (haptens) that are not in themselves antigenic was a major step forward.

In 1972, Chopra described the first RIA for T₄, (T₄RIA), a double antibody method, which allowed its measurement in unextracted serum. The procedure used a first antibody produced in rabbits by immunisation with human thyroglobulin and a second antibody of goat anti-rabbit gamma-globulin. The assay was set up in the presence of 8-anilino-1-naphthalene-sulphonic acid (ANS) to mobilise T₄ from its binding with TBG, and barbital buffer to inhibit binding of T₄ to TBPA. Since then a number of different methods have been applied to the RIA of human T₄.

In 1970, Brown, Ekins, Ellis and Reith reported the production of specific T₃ antibodies and subsequently, the first sensitive and precise RIA for T₃ (T₃RIA) in serum extracts was developed by Brown, Ekins, Ellis and Williams (1971). Eastman, Corcoran, Ekins, Williams and Nabarro (1975) reviewed the literature on the

development of methods for assaying T3 in man. They concluded that T3RIA had proved to be a precise and reliable method for the detection of thyroid dysfunction.

Clinical studies. Techniques for RIA of T4 and T3 are applicable to the cat provided the methods are modified to allow for the much lower concentrations of circulating total T4 and T3 in this species compared with man. Data on studies in the literature are summarised in Table 3.4.

Reap, Cass and Hightower (1978) were the first workers to report details of total T4 and T3 concentrations by RIA in "normal" cats in a study of ten animals (further details unspecified). Anderson and Brown (1979) reported a study of the total T4 concentrations of 92 healthy animals. Both groups used commercially produced RIA kits (Tetra-Tab RIA, Nuclear Medical Laboratories and ^{125}I -T3 RIA kit, Diagnostic Products Corporation; Immuntube, Smith Kline, respectively), which were designed for use with human serum. Both groups recognised the particular problems in applying these kits to assay the low levels of thyroid hormones in cats and attempted to circumvent them by doubling the volume of serum used. None of the assays was optimised for the cat and standards were prepared in buffer.

Reimers, Cowan, Davidson and Colby (1981), reporting on commercially available coated-tube RIAs for T4 and T3 (Autopak, Micromedic Systems), produced for use with human plasma, described their validation for canine, feline and equine serum. Standards were lyophilised in human plasma, beginning at 0 and 12.9 nmol/l for T4 and 0 and 0.77nmol/l for T3 and continuing in doubling concentrations to 510nmol/l and 9.24nmol/l respectively. To

increase the assay sensitivities, additional low standards (4.3nmol/l for T4 and 0.26nmol/l for T3) were produced by diluting the lowest standards supplied, 1:2 with buffer. Specificity of both assays was indicated by: (i) sample results falling in parallel with the standard curve on sample dilution, (ii) a lack of cross-reactivity for other iodothyronines tested, and (iii) appropriate biological responses in sera from seven cats stimulated with TSH, and several animals monitored post-thyroidectomy. Satisfactory accuracy was indicated by quantitative recovery of added analyte, for T4 using canine and equine but not feline serum, and for T3 using feline serum. Human and canine but no feline quality control samples were included in each assay. Sensitivity was evaluated by determining the intercept of the standard curves with the lower 95 per cent confidence limit of percentage of binding by the Bo standards, the mean of ten assays being 2.2nmol/l for T4 and 0.22nmol/l for T3. Within and between-assay precision figures were published for both T4 and T3 for canine and human, but not for feline, pools.

The authors did not report normal ranges for T4 or T3 concentrations for cats in this paper but values were subsequently published by Reimers (1982) and Reimers (cited by Randolph and Jorgensen, 1984). No further details were given but presumably these ranges were derived from the assays described above.

Hoenig, Goldschmidt, Ferguson, Koch and Eymontt (1982), using unspecified modifications of the double antibody RIA of Chopra (1972), reported the mean total T4 concentration of 51 healthy cats. Using unspecified modifications of the double antibody RIA of Liebllich and Utiger (1972), the same authors reported the total T3 concentration of 44 healthy animals.

Peterson, Keene, Ferguson and Pipers (1982) employed double antibody techniques for T4 and T3 modified by using charcoal-extracted, pooled serum from normal cats to produce T4 and T3 depleted serum for addition to control and standard tubes. The T4 and T3 ranges of an unspecified number of cats were reported but no further details were given. Peterson, Kintzer, Cavanagh, Fox, Ferguson, Johnson and Becker (1983b) used the same techniques in 67 animals and reported reference ranges.

Hoening and Ferguson (1983) gave further details of their modification of Chopra's double antibody RIA for T4 and reported the normal range for 67 animals. The first antibody had a cross-reactivity of 0.3 per cent for T3 and two per cent for rT3. They also included activated charcoal hormone-depleted serum in standard tubes in an attempt to equalise protein concentrations with samples. Dilutions of hyperthyroid cat sera to 75 per cent, 50 per cent and 25 per cent using hormone-depleted serum gave concentrations of 77.7 ± 2.2 per cent, 56.1 ± 1.9 per cent and 23.7 ± 1.3 per cent respectively (n=6) of the undiluted sera. Low, medium and high concentration recovery experiments gave results of 107.6 ± 6.0 per cent, 104.2 ± 5.1 per cent and 86.3 ± 5.4 per cent (n=8) respectively. The within-assay variation was 6.8 per cent. The working range of the assay was 2.6 to 644nmol/l but standard values were not stated.

Peterson et al. (1983b) used commercial solid phase techniques (Micromedic T4 and T3 RIA Kits, Micromedic Systems) and evaluated the serum total T4 and T3 concentrations of 135 normal cats but assay methodologies were not reported.

Donne and Wildgoose (1984) investigated commercially available, solid phase, magnetic antibody RIA (T4 and T3 MAIA Kits, Serono Diagnostics), designed for the assay of T4 and T3 in human plasma but modified for use with cat serum. The original standards were supplied in human serum and for T4 ranged from 0 and 25.7nmol/l to 411.8nmol/l and for T3 from 0 and 0.39nmol/l to 12.32nmol/l. In an attempt to obtain greater accuracy for the low serum T4 concentrations found in the cat, an extra standard of 12.9nmol/l was prepared and the highest standard reduced to 51.5nmol/l. In an investigation of 40 normal cats, the T4 range was quoted as 19.7 nmol/l to 68.3nmol/l. The authors did not explain how they were able to read values above the highest standard. No modifications were made to the T3 assay, the range being 0.46 to 1.71nmol/l.

Data on validation of the assay were presented in detail. Cross-reactivity of T4 antibody for T3 in extracted cat serum was 6.2 per cent, with that of T3 antibody for T4 being 0.28 per cent. Specificity was investigated by parallelism, dilutions of feline sera resulting in linear decreases in T4 and T3 concentrations. Accuracy was evaluated by assessing the percentage recovery of known amounts of T4 and T3 which had been added to feline serum, the range for T4 being 93 to 107 per cent and ranges for different concentrations of T3 being 98 to 110 per cent and 99 to 107 per cent. Within-assay precision expressed as mean \pm sd was 24.5 ± 0.9 nmol/l for T4 (coefficient of variation (cv) was 3.4 per cent, n=20) and 1.60 ± 0.06 nmol/l for T3 (cv was 3.8 per cent, n=22). Between-assay precision also expressed as mean \pm sd was 23.7 ± 1.8 nmol/l for T4 (cv was 7.4 per cent) and 1.66 ± 0.9 nmol/l for T3 (cv was 5.2 per cent, n was not specified). The detection limits of the assays were found to be 2.6nmol/l for T4 and 0.15nmol/l for T3.

Kemppainen, Mansfield and Sartin (1984) used a commercially available T4 RIA kit (Canine Thyroxine, Diagnostic Products Corporation), validated for use with cat serum, to measure the total T4 concentrations of 12 healthy cats. Although few details were reported, they stated that inhibition curves produced by serial dilutions of cat plasma were parallel with the respective standards, that when "varying" amounts of T4 were added to a cat plasma pool an average of 96 per cent was recovered, and that the sensitivity of the T4 assay was 1.9nmol/l.

The only other reports of total T4 or T3 concentrations using RIA methods in healthy cats have referred to normal ranges without data on the numbers of animals studied, in clinical papers on feline hyperthyroidism or in review articles. These publications and the values reported are presented in Table 3.4.

Generally, there is good agreement for feline T4 RIA values in the literature, with an approximate mean of 30nmol/l. This is perhaps surprising in view of the variety of techniques used and the number of centres from which they have been reported. The values for T3 are more variable and fall into two groups with ranges of approximately 0.8 to 3.0nmol/l and 0.23 to 1.60nmol/l. For T3, there is a trend for lower values to be reported in later studies.

Effects of anticoagulants, haemolysis, storage, freezing and thawing and anaesthesia on thyroxine and triiodothyronine radioimmunoassay. Kubasik and Sine (1978) compared the T4 and T3 concentrations of human heparinised plasma and serum using a number of commercially available RIA kits and found significant differences between the results obtained by some of the techniques.

Reimers, McCann, Cowan and Concannon (1982) investigated the effects of storage, haemolysis and freezing and thawing on T4 concentrations of five canine blood samples as measured by a commercially available, solid phase RIA (Autopak, Micromedic Systems). They also compared T4 concentrations in ethylenediaminetetraacetic acid (EDTA) - treated and heparinised blood and serum. Concentrations of T4 did not change in whole or clotted blood stored for 72 hours at 4°C or room temperature (22°C to 26°C) and in serum stored for eight days at -20°C, 4°C or room temperature. Repeated freezing and thawing of serum and haemolysis also did not significantly affect the T4 concentration. The overall mean concentration of T4 of plasma from heparinised blood was lower ($P < 0.05$; mean \pm sd = 61.3 ± 8.0 nmol/l) than that in EDTA-treated plasma (66.9 ± 9.1 nmol/l) and serum (67.2 ± 8.6 nmol/l).

Donne and Wildgoose (1984) carried out similar investigations into T4 and T3 concentrations of an unspecified number of feline blood samples using a commercially available solid phase, magnetic antibody RIA (MAIA kits, Serono Diagnostics). They compared the T4 and T3 concentrations of heparinised plasma and serum. The effect of haemolysis was examined by the addition of packed red cells to serum samples to simulate mild, moderate and severe red cell breakdown. Storage effects were evaluated by assay of samples stored at 8°C and -20°C. After collection, samples were aliquoted, with one being stored at 8°C and assayed within eight hours and the second stored at -20°C and assayed after 48 hours. None of these variables produced significant differences in T4 and T3 concentrations.

In the same report, the effects of the steroid anaesthetic mixture of alphaxalone and alphadalone acetate (Saffan, Glaxovet) on serum T4 and T3 concentrations were evaluated. Blood samples were collected from an unspecified number of cats before, and at intervals of from two to 60 minutes after, anaesthesia. There were slight increases in post-anaesthetic T4 and T3 concentrations but these were not statistically significant.

Total serum thyroxine and triiodothyronine concentrations in healthy cats by unspecified methods

Data from studies in which methodological details were not given are shown in Table 3.5. In some instances, the number of cats investigated was also not stated. The values of T4 reported are in the region of the highest concentrations reported by RIA, while the single mean T3 appears substantially higher than other reported values.

Free thyroxine index (FT4I)

Method principle. As stated previously, it is the free or unbound fraction of the plasma T4 which is able to leave the intravascular space, and take part in the production of metabolic effects at the tissue level. It follows, therefore, that FT4 is a more accurate indicator of thyroid status than total T4. The very low FT4 concentrations, are, however, technically difficult to measure. Although simple kit methods have recently become commercially available for FT4 in humans, the FT4 concentration in cats has not been reported.

In human medicine, a widely used solution to this problem has been to calculate a plasma free thyroxine index (FT4I) which is proportional to the FT4 concentration. The FT4I is a product of the

total T4 concentration and the T3 uptake. In effect, the total T4 is corrected for the number of unsaturated TBG binding sites, the latter being measured as the T3 uptake. In man, the FT4I is valuable in patients with acquired TBG excess e.g. in pregnancy or on oestrogen treatments. However, the test is of less use in patients with non-thyroidal illness or hereditary TBG excess or deficiency and adds little diagnostically to the majority of patients with normal TBG levels (Toft, Campbell and Seth, 1981).

Clinical study. Kallfelz and Erali (1973) are the only workers to have reported FT4I values in cats. Their data are presented in Table 3.6. They measured both the total serum CPBT4 concentration and the T3 uptake by commercially available kit methods (Tetrasorb-125 and Triosorb-125, respectively, Abbott Radiopharmaceuticals). The mean FT4I of 14 healthy cats derived from the product of total T4 in ug/100ml and T3 uptake expressed as a decimal, was 1.36. They commented that as the T3 uptake test did not seem responsive to changes in thyroid gland status in the dog, the FT4I in this species only reflected alterations occurring in the serum T4 concentration. They made no comment about cats. However, if cats do not possess a high affinity thyroid hormone binding protein comparable to human TBG, the situation is likely to be similar.

Assay of thyroid stimulating hormone

In human medicine, the major value of plasma basal TSH measurements is in the investigation of suspected primary hypothyroidism. Here, the test is a very sensitive indicator of thyroid failure, the TSH concentration commonly being elevated some two to three months before total T4 levels are depressed. Partial

thyroid failure can also result in an elevated basal TSH which serves to maintain normal plasma thyroid hormone concentrations and euthyroid status (Toft, Campbell and Seth, 1981). In secondary and tertiary hypothyroidism, the TSH concentration is low to normal.

TSH measured by RIA is undetectable in the plasma of more than 90 per cent of hyperthyroid patients but the same is true of 20 per cent of euthyroid humans. Thus, it is not possible to differentiate between these states using plasma basal TSH measurements (Toft, Campbell and Seth, 1981).

TSH is a species specific polypeptide. Attempts to measure canine TSH using a human TSH RIA were unsuccessful (Chastain, 1978). However, Nachreiner (1981a) reported that an RIA kit for measuring human TSH gave reliable values in dogs. Quinlan and Michaelson (1981) reported on an homologous, double antibody RIA for canine TSH which showed slight cross-reactivity with ovine, but not with human, bovine or rat TSH. It is, therefore, rather surprising that an earlier reference (Knigge and Joseph, 1971) reported assaying TSH extracted from cats' pituitary glands by modifying the method of McKenzie (1958) used to measure human TSH. In 19 normal cats, the mean pituitary TSH concentration was 13.4mu/mg.

Because of the lack of cross-reactivity between feline TSH and that from other species, and the lack of pure feline TSH for preparation of antisera and radiolabel for use in RIA, the levels of TSH in feline plasma have not been reported.

3.8 METHODS USED TO ASSESS NORMAL FELINE THYROID FUNCTION

- RADIONUCLIDE STUDIES

Introduction

The ability of the thyroid gland to concentrate and organify

iodide and subsequently release iodothyronines established an important role for radionuclide studies in the investigation and management of thyroid disease. Some techniques have been applied to the cat. Early tests were confined to research into thyroidal iodide uptake and conversion ratio, but with the recent identification of feline hyperthyroidism, more detailed investigations into radioiodide uptake, gamma camera imaging and rectilinear scanning have been carried out in normal cats for the purpose of comparison with thyrotoxic individuals.

Radioactive iodide (^{131}I , ^{125}I or ^{123}I), like stable iodine (^{127}I), is trapped and concentrated in the thyroid follicular cells by the iodide pump. It is subsequently incorporated into thyroglobulin which is stored in the follicular colloid, T₄ and T₃ being released by proteolysis. Daniel, Gale and Pratt (1962) discussed the advantages of using ^{125}I over ^{131}I for radionuclide studies in cats. They presented data which showed that the low energy radiations of ^{125}I made possible sensitive quantitative assessment at a relatively low radiation dose in vitro. However, in vivo counting of ^{125}I was less convenient. The longer half-life (27 days for ^{125}I compared with 8.1 days for ^{131}I) is a disadvantage for clinical work although it may be helpful in non-survival experiments.

$^{99\text{m}}\text{Tc}$ -Pertechnetate ($^{99\text{m}}\text{TcO}_4$), a radioisotope derived from molybdenum, is trapped and concentrated like iodide within thyroid follicular cells. However, it is neither incorporated in thyroglobulin nor stored in the thyroid (Pineda, Clauria, Rocha and Harbert, 1979) and thus reflects only the trapping mechanism of the thyroid gland. Its rapid uptake and short isotopic half-life (6.0 hours) give it considerable advantage for scintiscan work.

Thyroidal radioiodine uptake (Thyroidal RIU)

Method principle. The test uses the principle that the administered radionuclide is concentrated by the thyroid in a similar way to dietary iodide. The RIU therefore directly assesses the functional status of the thyroid. Generally, the higher the RIU, the more active is the thyroid (Peterson, 1984). Because $^{99m}\text{TcO}_4$ is not concentrated by the thyroid and because ^{125}I has the disadvantage of a longer half-life, ^{123}I or ^{131}I are commonly used. RIU is usually expressed as a percentage of the administered dose.

Although the estimation of thyroidal RIU is usually made 24 hours after the oral administration of radioiodine (Luick, 1971), the time of maximum uptake of ^{131}I in the cat has been variably reported as 72 hours (Knigge, 1961) and 96 hours (Bard and Woods, 1960).

Clinical studies. Reports of the use of this test in healthy cats are summarised in Table 3.7.

Knigge (1961) determined both the in vivo and the excised thyroid RIU in 40 normal cats, 72 hours after the intraperitoneal administration of 25 μCi of carrier-free ^{131}I . At this time, animals were anaesthetised and the right thyroid removed surgically. The excised thyroid RIU was determined using a lead-shielded scintillation crystal. Immediately after hemi-thyroidectomy, the in vivo uptake of the intact lobe was also determined. The mean value \pm sd of the latter was 6.6 ± 2.0 per cent of the administered dose. This was a mean \pm sd of 13.0 ± 0.5 per cent less than the excised uptake value.

The only other reports of thyroid RIU in normal cats have been for comparison purposes with hyperthyroid individuals. Peterson, Becker and Hurley (1980), Peterson, Becker, Hurley and Ferguson (1981), Peterson et al. (1982) and Peterson and Becker (1983) gave no further information as to techniques or numbers of animals studied.

Hoenig et al. (1982) and Peterson et al. (1983b) described the equipment they used to carry out RIU. Both assessed RIU at 24 hours but the former group also compared this to uptake at six hours.

Overall, the studies using ^{131}I show good agreement for uptake at 24 hours. The study by Hoenig et al. (1982) using ^{123}I shows higher values but the reasons for this are not clear.

Radioiodine retention and excretion

The metabolism of radioiodine has been investigated by measuring its retention in the body or in the blood, or by monitoring its urinary excretion. These are very crude techniques but were used in early investigations to give some measure of feline thyroid function.

Nelson, Wolfangel and Stara (1966) administered aqueous $\text{Na } ^{131}\text{I}$ intravenously to 12 cats and assayed radioactivity in the whole body and blood samples "regularly". Whole blood displayed the same general downward trend as exhibited by the whole body retention curves. The cat appeared to retain a high proportion (80 per cent) of the initial ^{131}I dose at 24 hours. Further details were not given.

Randall and Littschwager (1967) determined the percentage urinary excretion of ^{131}I in ten healthy cats at monthly intervals for 20 months. One to 3 μCi of $\text{Na } ^{131}\text{I}$ was injected

intramuscularly into each animal, and an identical volume was retained for comparison purposes. The urine of each cat was collected for five days, individually pooled, aliquoted and counted in a scintillation gamma counter. Identical volumes of the solution retained for comparison were also counted. After calculating the percentage of the injected dose of Na ^{131}I that was in the total volume of urine, the percentage ^{131}I retention was determined by subtraction from 100. The values varied between individuals and on the time of year, results being from -2.4 per cent to 58 per cent. This remarkable variation and the fact that the authors appeared to make no allowance for radioiodine loss in the faeces meant that little could be deduced from the data.

Protein-bound radioiodine and the conversion ratio

Method principle. The rate at which radioiodine-labelled thyroid hormone is secreted into the circulation after the administration of a tracer dose of radioiodine (e.g. ^{131}I) is related to the functional state of the gland. In order to estimate the amount of labelled hormone in the plasma in the presence of inorganic radioiodide, use is made of the strong binding of T₄ to plasma proteins. The hormonally active iodine is precipitated with plasma proteins using, for example, trichloroacetic acid, and is termed the plasma protein-bound ^{131}I (PB ^{131}I). The inorganic iodide can be removed from plasma using an anion exchange resin (Zieve, Vogel and Schultz, 1956) which does not absorb the protein-bound forms. Thus, the radioactivity of the PB ^{131}I and of the inorganic iodide may be evaluated separately.

Clark, Moe and Adams (1949) introduced the term 'conversion ratio' (PB ^{131}I -CR) for a given quantity of plasma, which was defined

by the equation: Conversion ratio (per cent) =

$$\frac{\text{Plasma protein radioactivity}}{\text{Total plasma radioactivity}} \times 100 = \frac{\text{PB } ^{131}\text{I}}{\text{PB } ^{131}\text{I} + ^{131}\text{I}} \times 100$$

In human hyperthyroids, a greater amount of labelled hormone is found in the plasma after 24 hours than in euthyroids (Luick, 1971) and the conversion ratio rises.

Clinical study. Knigge (1961) reported the thyroid conversion ratio of 40 normal cats, 24 hours after the intraperitoneal injection of 25u Ci of carrier-free ^{131}I , to be a mean \pm sd of 27.6 ± 7.3 per cent. This test has not been evaluated in feline hyperthyroidism.

Estimate of the rate of release of radiolabelled thyroid hormones

Randall and Littschwager (1967) attempted to measure the rate of release of radiolabelled T4 in 15 healthy cats. Beginning seven days after the intramuscular injection of one to three uCi Na ^{131}I to each cat, daily determinations of the radioactivity in the neck region were made for three weeks, or until the counts fell to approximately ten times the background. To determine what they called "an estimate of the average daily release of thyroxin from the thyroid gland" the three largest intervals were selected for evaluation, e.g. in 21 days, three 18 day intervals would be available (one to 18, two to 20 and three to 21). After corrections for background and decay, the rates of gamma irradiation on days one and 19 were used to obtain the constant a in the equation:

$$\log_{10} N_t = -at + c$$

where N_t is the rate of gamma irradiation on day t. The average daily release (i) was then calculated from:

$$i = 1 - 10^{-a}$$

The mean daily release was thus calculated for each of the 18 day intervals. The mean of the three scores (\bar{i}) was an estimate of the percentage of the organically bound ^{131}I existing on any one day that was absent 24 hours later.

This value is clearly not an estimate of the release of T4 as it makes no allowance for T3 or other radiolabelled iodothyronines. The values they obtained varied from 1.4 per cent to 9.1 per cent per animal for each period tested.

This test should not be confused with the thyroxine secretion rate which has usually been determined in domestic species by the amount of exogenous L-T4 necessary to inhibit the release of ^{131}I by the thyroid i.e. by T4 substitution (Kaneko, 1980a).

Thyroid imaging

Method principle. Scanning the thyroid with a highly collimated camera after administration of a suitable radionuclide serves to delineate areas with relatively high or low uptake. The thyroid scan is useful in hyperthyroidism to determine unilateral or bilateral lobe involvement, abnormal thyroid lobe position or metastasis. The radionuclides most commonly used for thyroid imaging are isotopes of iodine (^{131}I and ^{123}I) and technetium-99m as pertechnetate ($^{99\text{m}}\text{TcO}_4$). Similar images are produced by each. However, in the cat, as in man, there are several advantages of using $^{99\text{m}}\text{TcO}_4$ instead of radioiodine (Peterson and Becker, 1984).

Because of the rapid uptake of $^{99\text{m}}\text{TcO}_4$, the imaging procedure can begin only 20 minutes after administration, as opposed to four and 24 hours for ^{123}I and ^{131}I , respectively. ^{131}I has a long physical half-life (8.1 days) and emits high-

energy gamma rays (364 keV) which are inefficiently collimated by the scintillation camera. Also, ^{131}I emits beta particles that are not detected by the camera but increase total body and thyroid radiation exposure (Johnson, 1978). In contrast to ^{131}I , ^{123}I has a short physical half-life (13.3 hours), emits low-energy gamma rays (159 keV) well-suited for scanning and has no beta emission (Pineda et al., 1979). Regrettably, it is very expensive. Pertechnetate has a short physical half-life (six hours), emits low-energy gamma rays (140 keV), has no beta emission and gives the lowest radiation dose to the thyroid gland (Dos Remedios, Weber and Jasko, 1971). Thus, higher doses of $^{99\text{m}}\text{TcO}_4$ may be given to patients than of radioiodine and thyroid imaging may be completed more rapidly.

Clinical studies. Hoenig et al., (1982) carried out rectilinear thyroid scanning of five normal cats after the oral administration of 100 uCi ^{123}I . Animals were premedicated with atropine and sedated with ketamine. A five inch rectilinear scanner with a fine focus collimator was employed, using a 140 to 180 keV window. The resulting scan revealed homogenous uptake of radioiodide by both lobes.

Peterson et al., (1983b) performed thyroid scans on 13 clinically normal cats. Twenty minutes after the intravenous administration of 0.5mCi $^{99\text{m}}\text{TcO}_4$, the cats were sedated with ketamine and scanned, using a scintillation gamma camera with a pinhole collimator. The thyroid glands showed uniform distribution of radioactivity throughout both lobes. The lobes were symmetrical in position and size and an isthmus was not visible.

Peterson and Becker (1984) compared the results of radionuclide imaging using either $^{99m}\text{TcO}_4$ or ^{131}I as described above, in 13 normal cats. Imaging (16,000 counts, 140 keV setting) was carried out with a scintillation gamma camera and a 5mm pinhole collimator. Scans were also performed 24 hours after intravenous administration of 10 uCi to 25 uCi of ^{131}I . Here, 8,000 counts were imaged at the ^{131}I peak setting (364 keV) with the same scintillation camera and pinhole collimator. All cats showed uniform distribution of radioactivity throughout both lobes in images obtained with both radionuclides. As described previously, the lobes were symmetrical in position and size and no isthmus was visible. The quality of the scans obtained with $^{99m}\text{TcO}_4$ was consistently equal or superior to that of those obtained with ^{131}I .

These results were also summarised by Peterson (1984).

3.9 METHODS USED TO ASSESS NORMAL FELINE THYROID FUNCTION

- DYNAMIC FUNCTION TESTS

The thyroid stimulating hormone stimulation test

Introduction. Administration of exogenous (usually bovine) TSH followed by the measurement of some parameter of thyroid function provides important information about thyroid status. In veterinary medicine, the test has been most widely applied to the diagnosis of primary hypothyroidism in dogs (Belshaw, 1983). In such cases, there is no significant increase in serum T4 concentration after TSH administration. The test will differentiate hypothyroxinaemia due to non-thyroidal illness or drug administration (responses similar to normal) from primary hypothyroidism (Belshaw, 1983). It may also be used to differentiate secondary and tertiary hypothyroidism from

the primary form of the disease (Chastain, Riedesel and Graham, 1979). In secondary and tertiary hypothyroidism, one dose of TSH usually leads to serum increases of T4 similar to those seen in non-thyroidal illness or as a result of drug administration. A small percentage of cases may require repeated administration of TSH for three consecutive days before a significant increase is seen (Ferguson, 1984), as prolonged TSH deficiency may lead to glandular atrophy, limiting the thyroid's ability to respond to exogenous TSH (Rijnberk, 1971).

Method principle. As discussed in Section 3.6, TSH stimulates a number of steps in thyroid hormone synthesis. Clinical application of the test relies on the measurement of one of these physiological alterations.

In human medicine, the response to TSH has been gauged by increases in basal metabolic rate (Thompson, Taylor, Thompson, Nadler and Dickie, 1936; Querido and Stanbury, 1950), thyroidal ^{131}I uptake at various times after radioiodine administration (Perloff, Levy and Despopoulos, 1951; Schneeberg, Perloff and Levy, 1954; Burke, 1968), serum PB1 or PB ^{131}I concentrations (Jefferies, Levy, Palmer, Storaasli and Kelly, 1953; Einhorn and Larsson, 1959), urinary excretion of ^{131}I (Querido and Stanbury, 1950), and by the use of scintigraphy (Dos Remedios, Weber and Jasko, 1971). The technique used to be of diagnostic value in differentiating between primary and secondary hypothyroidism (Toft, Campbell and Seth, 1981) and in delineating the state of low thyroid reserve (Jefferies, Kelly, Levy, Cooper and Prouty, 1956). However, a single basal plasma TSH concentration provides the same information and the TSH stimulation test is now obsolete in human

medicine (Toft, Campbell and Seth, 1981). In the absence of assays for canine TSH, however, the technique is still regarded by many veterinary endocrinologists and dermatologists as the most convenient method for the definitive diagnosis of canine hypothyroidism (Oliver and Waldrop, 1983). The test is usually used in conjunction with the measurement of T4 by RIA. Because the majority of T3 is produced by mono-de-iodination of T4, serum T3 determination after TSH stimulation is diagnostically unreliable. (Nesbitt, 1983).

In cats, the TSH stimulation test has been used in conjunction with chromatography of radiolabelled serum, the T3 uptake test and T4 determination by CPB or RIA.

Clinical studies. Literature reports of the TSH stimulation test in healthy cats are presented in Table 3.8.

The earliest studies (Daniel, Excell, Gale and Pratt, 1962; Taurog, Porter and Thio, 1964) were physiological rather than clinical and have been discussed in detail in Section 3.6.

Ling, Lowenstine and Kaneko (1974) gave the first report of the use of TSH stimulation with biochemical tests of thyroid function in ten female cats, none of which was older than two years. There was no significant difference in the T3 uptake test before and after administration of TSH but T4 concentrations by CPB were significantly elevated post-stimulation.

Hoenig and Ferguson (1983) reported the results of a detailed investigation into thyroid functional reserve in 19 healthy cats as determined by response to TSH. Nine cats were male, ten female, 12 were American short-hairs and seven were Siamese. Details of the T4 RIA used are discussed in Sections 3.7, 4.1 and 4.7. Doses of TSH

used varied from 0.05 to 1.00 iu/kg and animals were sampled before and two, four, six, eight, ten, 12, 24, and 48 hours after administration. Greatest stimulation was seen with the highest TSH dose used. With all TSH doses, the peak serum T4 concentration was found at six hours except with 0.05 iu/kg which gave a peak at four hours. There was a linear correlation between the T4 concentration at six hours post-TSH and the logarithm of the TSH dose ($P < 0.001$), and between the absolute increase in serum T4 after TSH administration and the logarithm of the TSH dose ($P < 0.001$). The ratio of post-TSH T4 concentration divided by the pre-TSH T4 concentration, the most commonly used index of TSH stimulation in dogs, correlated less well with the logarithm of the TSH dose ($P < 0.01$). The authors concluded that in euthyroid cats, intravenous administration of 1 iu/kg TSH should lead to a T4 concentration of greater than 51.5 nmol/l at six hours after administration, or to an increment in the serum T4 of at least 32.3 nmol/l.

Kemppainen, Mansfield and Sartin (1984) carried out investigations into the serum T4 response to bovine TSH in 12 healthy cats (five neutered females and seven neutered males). The cats were divided randomly into three groups of four. After blood sampling, TSH was administered intramuscularly at a dose of 2.5 iu/cat (0.55 to 0.69 iu/kg) to one group, and 0.1 iu/kg (0.38 to 0.45 iu per cat) to a second group. Cats in the third group were administered the vehicle only and acted as controls. Further blood samples were collected four, eight, 12 and 24 hours later. T4 samples were determined by RIA as discussed in detail in Section 3.7. The response of serum T4 to 0.1 iu/kg TSH was not significantly different from controls. However, the authors

concluded that an intramuscular dose of 2.5 iu TSH per cat would more than double the serum basal T4 and give a concentration of greater than 38.6 nmol/l at eight and/or 12 hours after administration.

The other reports of the use of the TSH stimulation test in healthy cats either have given only brief details of regimes and results for comparison with those found in specific disease entities (FEA and hyperthyroidism), or been in review papers. Information from these publications is presented in Table 3.8.

Generally there is good agreement for feline T4 responses to TSH, with most authors citing an increase to at least twice the basal concentration. This is perhaps surprising in view of the variety of doses of TSH and routes of administration used.

The thyrotropin releasing hormone response test

Method principle. TRH, released by the hypothalamic neurones, stimulates synthesis and release of TSH by the thyrotrophs of the anterior pituitary. TSH subsequently stimulates production of T4 and T3 by the thyroid. Elevations of plasma T4 and T3 after TRH administration indicate a functionally intact pituitary-thyroid axis.

In human medicine, measurements of plasma TSH before and after the intravenous injection of TRH have proved to be extremely valuable in the investigation of patients with suspected hyperthyroidism. In normal subjects, there is a marked increase in plasma TSH concentration after TRH, whereas in hyperthyroidism, due to suppression of the thyrotrophs, there is no significant rise (Toft, Campbell and Seth, 1981). In primary hypothyroidism, the basal plasma TSH concentration is raised and no additional information is gained by performing a TRH test. However the test is of value in

differentiating between pituitary and the rare hypothalamic forms of hypothyroidism. In pituitary hypothyroidism, there is little or no TSH response whereas in the hypothalamic form of the disease, there may be a delayed (compared to euthyroid individuals) TSH response (Toft, Campbell and Seth, 1981).

Because TSH assays are not available for cats, the response to TRH must be determined by assaying T4 and T3.

Clinical study. Lothrop, Tamas and Fadok (1984) are the only workers to have reported the use of the TRH response test in cats (Table 3.9). A doubling of serum basal T4 concentrations was seen at four hours after stimulation with all doses of TRH used. There was no change in the size of the T4 response as the dose of TRH increased from 0.02 mg/kg to 10 mg/kg. Side effects of TRH administration were observed at doses above 0.02 mg/kg and included salivation, urination, defaecation, vomition, pupillary constriction, tachycardia and tachypnoea. These result from the central cholinergic properties of TRH (Yarborough, 1983). By withholding food for three to six hours before TRH administration, vomition was prevented in most cats.

The authors concluded that the TRH stimulation test might be valuable in evaluating pituitary, as well as thyroid-based, dysfunctions of the feline thyroid gland. They recommended a test procedure of serum total T4 determination before, and six hours after, the intravenous administration of 0.1 mg/kg TSH (although this dose of TRH was not used in cats in their study).

3.10 EFFECTS OF AGE, SEX AND BREED ON THYROID FUNCTION OF HEALTHY CATS.

Introduction

Little has been published on the effects of age, sex and breed on the thyroid function of healthy cats. Details of the methodologies and results of the reports here discussed are presented in Section 3.7 and in Tables 3.2, 3.3, 3.4 and 3.6.

Effects of age

Wilson, Dickson and Frost (1961) reported no age-related differences in the RBC T3 uptake test in cats but studied only five animals.

Kallfelz and Erali (1973) investigated the effects of age using the T3 uptake test, T4CPB and the FT4I in five cats aged ten weeks, four aged one year and five that were more than one year old. There was a trend towards decreasing concentrations of T4 with increasing age but this was not statistically significant.

Ling, Lowenstine and Kaneko (1974) evaluated the T3 uptake test in 39 and the T4CPB in 40 healthy cats. Sixteen animals were less than one year old, 17 were from one to two years and five (T3 uptake) and six (T4CPB) were older than two years of age. No animal examined was more than five years old. There were no significant age effects for either of the tests.

Using the T3 uptake test and T4 RIA, Anderson and Brown (1979) found no effects of age on thyroid function of 92 healthy cats varying between five months and 15 years but the actual numbers in each group were not stated.

Thus, no effects of age on feline thyroid function have been identified.

Effects of sex

Wilson, Dickson and Frost (1961) found no significant difference in the RBC T3 uptake test according to sex of only five animals, but did not present data.

Using CPB, Ling, Lowenstine and Kaneko (1974) studied the T4 concentration of 31 female and nine male healthy cats. There was no significant sex difference in total T4 concentration.

Bigler (1976b) found that the T4 concentration, as determined by CPB, of 55 female cats (one of which was ovariohysterectomised) was significantly higher ($P < 0.01$) than that of 55 male cats (seven of which were castrated). The effects of neutering were not reported.

Using RIA, Reap, Cass and Hightower (1978) failed to identify any sex differences in total T4 and T3 concentrations of ten healthy cats but they did not state whether any animals had been neutered and detailed data were not presented.

Anderson and Brown (1979), also using RIA, failed to identify any significant difference between the total T4 concentrations of 52 male cats (28 entire and 24 castrated) and 40 female cats (23 entire and 17 ovariohysterectomised). However, they reported that "a significant difference was found for serum T4 values of intact females and those of altered females" but they presented no statistical evidence for this assertion. Moreover, the large sds associated with the ranges lead to some doubts about the validity of their conclusions (Table 3.10).

Donne and Wildgoose (1984) presented numerical data on the total T4 and T3 concentrations of 40 "mixed" cats (15 male and 25 female) as measured by solid phase, magnetic antibody immunoassay

(Table 3.10). Female cats had a slightly higher mean T4 and a slightly lower mean T3 but the authors did not further analyse these data according to sex.

The findings from these reports are contradictory, with only one (Bigler, 1976b) describing a difference for T4 concentrations between male and female animals. These discrepancies may result from the small number of observations in some of the studies.

Effects of breed

Wilson, Dickson and Frost (1961) reported no differences in the RBC T3 uptake test according to breed of five cats but they presented no data.

Ling, Lowenstine and Kaneko (1974) found no significant difference for the T3 uptake test between 17 domestic short-haired, seven domestic long-haired and 11 Siamese cats (Table 3.2). Using CPB, however, they found a significantly higher concentration of total T4 in Siamese (n = 11) compared with domestic long-haired cats (n = seven) but no difference between T4 in either of these breeds and domestic short-haired cats (n = ten) (Table 3.3).

3.11 FELINE HYPERTHYROIDISM

Introduction

Feline hyperthyroidism (thyrotoxicosis) is a multisystemic disorder resulting from excessive circulating concentrations of T4 and T3. Although reports of suspected cases appeared sporadically in the early veterinary literature, the first cases were only confirmed in 1979 by Cotter and by Peterson, Johnson and Andrews. Feline hyperthyroidism has also been produced experimentally.

This section reviews these reports.

Naturally-occurring cases of feline hyperthyroidism

Causes. Benign thyroid tumours are the commonest cause of feline hyperthyroidism (Peterson, 1984). However, there is considerable uncertainty about the classification of such tumours in cats and Lucke (1964) was doubtful whether a true distinction should be made between what she termed "nodular (adenomatous) goitre" and single or multiple adenomas, except on the basis of size. Thus, while some authors have attempted to make the distinction (eg. Theran and Holzworth, 1980; McMillan and Scherding, 1981; Theran, 1981; Martin and Capen, 1983; Turrel, Feldman, Hays and Hornof, 1984), many workers have reported the pathological changes simply as "thyroid adenomas (adenomatous hyperplasia)" (Peterson et al., 1982; Peterson et al., 1983b; Birchard, Peterson and Jacobson, 1984; Bond and Fox, 1984; Peterson, 1984; Peterson and Becker, 1984). While early reports of small numbers of cases suggested that unilateral thyroid enlargements were more common (Cotter, 1979; Peterson, Johnson and Andrews, 1979), later studies in which thyroid scans were performed, confirmed bilateral hyperfunction in between 71 per cent and 79 per cent of affected animals (Hoenig et al., 1982; Peterson et al., 1983b).

Thyroid carcinoma, the primary cause of hyperthyroidism in the dog, rarely causes hyperthyroidism in the cat (Peterson, Becker and Hurley, 1980; Peterson, 1981; Peterson et al., 1981; Theran, 1981; Hoenig et al., 1982; Cotter, 1983; Fox, 1983).

Accessory or ectopic thyroid tissue may occasionally be involved in the production of hyperthyroidism (Noxon et al., 1983; Olsen, 1983).

Incidence and prevalence. Until recently, feline thyroid lesions were considered to produce no clinical signs and to be incidental findings at post-mortem examination (Brodey, 1970; Capen, 1978). However, with the increased availability of thyroid hormone RIA, hyperthyroidism is now thought to be one of the most common feline endocrine disorders, with Peterson (1984) reporting the diagnosis in approximately one of every 300 cats examined at the Animal Medical Center, New York, USA. Between 98 per cent and 99 per cent of cases are caused by thyroid adenomas (adenomatous hyperplasia). The prevalence of functional thyroid carcinoma is approximately one to two per cent (Peterson et al., 1983b).

Unsubstantiated reports. Prior to T4 and T3 assay procedures being available to veterinary clinicians, a number of suspected cases of feline hyperthyroidism were reported.

Holzworth, Husted and Wind (1955) reported a case of arterial thrombosis and thyroid carcinoma in a domestic short-haired cat (discussed in detail in Section 3.12). Their initial diagnosis was hypothyroidism but retrospectively they considered the animal might have actually been hyperthyroid.

Meier and Clark (1958) diagnosed a single case of feline hyperthyroidism "according to the presence or lack of functional disturbances associated with thyroid changes" together with a low serum cholesterol concentration. Further details were not reported.

Lucke (1964) described a 14-year-old, neutered female cat which presented with weight loss, vomiting and panting. At post-mortem examination there was adenomatous goitre, left and right ventricular hypertrophy of the heart and metastatic calcification of the lungs, the superior mesenteric artery and the kidneys, suggesting

hyperthyroidism. A second cat also showed marked ventricular hypertrophy in association with adenomatous goitre, but thyroid function tests were not carried out in either case.

Leav, Schiller, Rijnberk, Legg and der Kinderen (1976) reported that, retrospectively, "some" of 52 cats with thyroid tumours had clinical signs which might have reflected an altered hormonal status. The PBI of one animal was elevated suggesting possible thyrotoxicosis but further details were not available.

Flecknell, Gruffydd-Jones, Brown and Kelly (1979) reported the ECG findings in a seven-year-old, neutered male, Russian blue cat. There was evidence of ventricular pre-excitation, and the authors suggested that this was similar to that found in the Lown-Ganong-Levene syndrome of man. At post-mortem examination, a thyroid adenoma was identified but the authors felt that this was a coincidental lesion. Thyroid hormone determinations were not carried out but it is likely that the arrhythmia present in this cat was secondary to hyperthyroidism.

O'Brien, Riley and Hagemoser (1980) reported details of an aged cat which showed clinical signs of hyperthyroidism and at post-mortem examination an atypical thyroid adenoma was identified. Serum thyroid hormone concentrations were not determined.

Thus, a number of cats with clinical features similar to hyperthyroidism have been described in the veterinary literature. The frequency with which the disease has been identified subsequently, suggests that many of these cats were indeed hyperthyroid.

Confirmed cases

Breed, sex and age predispositions. There is no breed predisposition for feline hyperthyroidism (Peterson, 1982, 1984). While the majority of cases have been reported in domestic short and long-haired cats, cases have also occurred in Siamese and Persians (Hoenig et al., 1982; Peterson et al., 1982; Peterson et al., 1983b) and a Russian blue (Peterson et al., 1983b). Similarly, there is no sex predisposition (Peterson, 1982; Hoenig, 1983). In a series of 135 affected animals, 68 were male and 67 were female (Peterson and Becker, 1984).

Hyperthyroidism is a disease of middle-aged to old cats (Peterson, 1982). The youngest yet recorded age at onset has been six years (Hoenig et al., 1982; Peterson et al., 1983b) and the oldest, 22 years (Theran and Holzworth, 1980; Theran, 1981). Hoenig et al. (1982) reported a mean age at onset of 11.8 years in a series of 24 cases and Peterson et al. (1983b) a mean age of 12.8 years in a series of 131 cases.

Historical and clinical features. The signs exhibited by cats with hyperthyroidism vary from mild to severe and are modified by the duration of the condition, the presence of concomitant abnormalities in other organ systems and the inability of a body system to meet the demands imposed by thyroid hormone excess (Peterson, 1984). Most affected individuals show evidence of dysfunction of a number of systems but occasionally disturbances in one predominate.

Table 3.11 lists the historical and clinical findings reported in the three, major, published, independent series of feline hyperthyroidism. They are largely in agreement with one another and with other smaller series and single case reports.

Physical appearance. Most hyperthyroid cats exhibit mild to severe weight loss. Skin changes consisting of mats, greasy seborrhoea (Holzworth et al., 1980), excessive shedding of hair (Peterson, 1984) and varying degrees of alopecia (Holzworth et al., 1980; Hoenig et al., 1982; Peterson et al., 1983b) are common. Changes in coat colour from black to rust-brown were recorded in one case (Jones and Johnstone, 1981). Affected cats may show an anxious, frantic, facial expression (Peterson, 1984).

Gastrointestinal signs. All authors agree that polyphagia is one of the most frequently seen symptoms of feline hyperthyroidism. However, although the cause is unknown, approximately 20 per cent of affected cats show short periods of decreased appetite which alternate with longer intervals of polyphagia (Peterson, 1982; Peterson et al., 1983b; Peterson, 1984). Other common gastrointestinal signs include vomiting, increased frequency of defaecation, diarrhoea and voluminous faeces (Peterson, 1982) and excessive flatus (Theran and Holzworth, 1980). Steatorrhoea may also be present (Peterson, Becker and Hurley, 1980; Peterson et al., 1981).

Nervous and muscular signs. Central nervous system abnormalities in hyperthyroid cats are evidenced by increased activity, restlessness, nervousness, irritability, pacing and excessive grooming (Holzworth et al., 1980; Hoenig et al., 1982; Peterson et al., 1983b). Muscular weakness is usually accompanied by severe muscle wasting (Peterson, 1984).

Cardiovascular signs. Numerous cardiovascular abnormalities have been recorded in hyperthyroid cats. Tachycardia with or without premature beats is common (Holzworth et al., 1980) with

rates up to 320 beats per minute (Liu, Peterson and Fox, 1984). There may be a prominent precordial impulse, apical systolic or holosystolic murmurs (Holzworth et al., 1980), gallop rhythm, cardiomegaly and congestive cardiac failure (Keene and Peterson, 1980; Peterson et al., 1982; Liu, Peterson and Fox, 1984). Three of ten cases had hyperaemia of the mucous membranes and the skin of the ears suggesting possible hypertension (Holzworth et al., 1980).

Apathetic hyperthyroidism. A form of the disease comparable with apathetic (masked) hyperthyroidism of man (Thomas, Mazzaferri and Skillman, 1970) occurs in approximately ten per cent of affected animals (Peterson et al., 1983b). These cases are depressed and weak and inappetant or anorexic rather than hyperactive and polyphagic. They may show ventral neck flexion. Severe cardiac abnormalities (arrhythmia, congestive cardiac failure) are common (Peterson, 1984).

Thyroid gland. Enlargement of one or both thyroid lobes is detectable by careful palpation in between 85 per cent (Peterson, 1984) and 100 per cent (Holzworth et al., 1980) of hyperthyroid cats. Affected glands are freely mobile and may become retro-tracheal (Holzworth et al., 1980; Theran and Holzworth, 1980) or intra-thoracic (Peterson et al., 1983b; Peterson and Yoshioka, 1983; Peterson and Becker, 1984). Anterior migration of intra-thoracic masses for diagnostic purposes may be made possible by holding sedated or anaesthetised animals by the hind limbs with the head pointing downwards (Peterson and Yoshioka, 1983).

Investigative procedures. A large number of diagnostic tests have been applied to the investigation of feline hyperthyroidism. These are described below. The pathomechanisms behind the changes are discussed in Chapter 10.

Radiology. Although Fox (1983) reported that thoracic radiographs of hyperthyroid cats were generally unremarkable, Bond and Fox (1984) commented that varying degrees of cardiomegaly may be present. In a number of series of cases, cardiomegaly has been reported in 25 per cent (Hoenig et al., 1982; Hoenig, 1983), 40 per cent (Peterson et al., 1983b), 45 per cent (Liu, Peterson and Fox, 1984) and 50 per cent (Holzworth et al., 1980) of animals radiographed. When the change is severe, there may be evidence of extracardiac abnormalities associated with heart failure such as pulmonary oedema, pleural effusion or both (Peterson et al., 1983b; Liu, Peterson and Fox, 1984; Cowell and Cowell, 1985). Fox (1983) reported that angiocardigraphy may fail to display abnormalities or may show mild to moderate hypertrophy of the left ventricular posterior wall.

Signs of pulmonic hyperinflation were evident in six of 24 cases (Hoenig et al., 1982) and moderate hepatomegaly in two of 10 others (Holzworth et al., 1980).

Electrocardiography. Electrocardiographic disturbances are common in hyperthyroid cats (Peterson et al., 1982). The most frequent changes are sinus tachycardia (rate greater than 240 beats per minute) and an increase in R wave amplitude in lead II (greater than 0.9 mV) suggestive of left ventricular enlargement (Peterson, Johnson and Andrews, 1979; Kruth, 1980; Peterson, Becker and Hurley, 1980; Peterson et al., 1981; Hoenig et al., 1982; Olsen, 1982; Peterson, 1982; Peterson et al., 1982; Bond, Fox and Peterson, 1983a; Fox, 1983; Fox, Tilley and Liu, 1983; Hoenig, 1983; Martin and Capen, 1983; Olsen, 1983; Peterson, 1983; Peterson et al., 1983b; Rochlitz, 1983; Bond and Fox, 1984;

Peterson, 1984). In the largest series (131 cases) of ECGs from hyperthyroid cats yet reported (Peterson et al., 1983b), 66 per cent showed tachycardia and 29 per cent increased R wave amplitude. However, numerous other changes were recorded including prolonged QRS duration (18 per cent), short Q-T interval (ten per cent), atrial premature complexes (seven per cent), left anterior fascicular block (six per cent), ventricular premature complexes, right bundle-branch block, first-degree atrioventricular block and second-degree atrioventricular block with ventricular escape complexes (two per cent each) and atrial tachycardia, ventricular tachycardia and bigeminy and ventricular pre-excitation (one per cent each). Almost all of these changes have been recorded variously by the authors cited elsewhere in this section and in small series or single cases reported by Holzworth et al., 1980; Keene and Peterson, 1980; McMillan and Scherding, 1981; Webb and Atwell, 1981; Noxon et al., 1983 and Peterson and Yoshioka, 1983.

Liu, Peterson and Fox (1984) reported on the electrocardiographic findings in 23 cats with hyperthyroid-associated hypertrophic cardiomyopathy. Seventy per cent showed tachycardia, 22 per cent left ventricular enlargement pattern, 13 per cent atrial premature complexes, nine per cent ventricular premature complexes and nine per cent left anterior fascicular block.

Echocardiography. Echocardiographic abnormalities are common in hyperthyroid cats, with Bond, Fox and Peterson (1983b) reporting changes in 29 of 30 animals examined. Because of hyperactivity, Bond, Fox and Peterson (1983a) administered ketamine hydrochloride prior to carrying out the procedure. Optimum M-mode

echocardiograms were obtained by placing the cats in left lateral recumbency, and pointing the transducer immediately off the right sternal border in the third to fourth rib interspace.

In these and other contributions (Fox, 1983; Bond and Fox, 1984), the most common abnormalities reported were left ventricular hypertrophy, aortic root and left atrial enlargement and increased parameters of contractibility (increased shortening fraction and velocity of circumferential fibre shortening).

Serum thyroid hormone concentrations. Resting serum total concentrations of both T4 and T3 are elevated above the normal range in most hyperthyroid cats (Peterson, 1982; Peterson et al., 1982; Peterson, 1983). The T4 concentration may be up to 14 times the upper limit of the reference range with a concentration of 696 nmol/l having been recorded (Peterson et al., 1983b; Peterson and Becker, 1984). The T3 concentration may be up to 17 times the upper limit of the reference range with a concentration of 15.40 nmol/l having been recorded (Peterson et al., 1983b; Peterson and Becker, 1984).

In a series of 131 cases, the T4 concentrations were elevated in all, whereas the T3 concentrations were in the normal range in four cases (three per cent) (Peterson et al., 1983b). These four cats showed only mild symptoms and signs of hyperthyroidism and had a mean T4 concentration of only 86.2 nmol/l (normal range was 10.3 nmol/l to 48.9 nmol/l). Other investigators have also reported normal concentrations of T3 in cats with hyperthyroidism (Holzworth et al., 1980; McMillan and Scherding, 1981). It is likely that these normal T3 concentrations would increase into the thyrotoxic range if the disorder were allowed to progress untreated (Peterson, 1982, 1983; Peterson et al., 1983b; Peterson, 1984).

Turrel et al. (1984) have suggested that concentrations of T4 and T3 should exceed three sds from the mean to confirm a diagnosis of hyperthyroidism in cases where these values are only marginally elevated.

Hoenig (1983) found that the ratio of total T3 concentration to total T4 concentration was increased in hyperthyroid cats.

Peterson (1982), Peterson et al. (1983b) and Peterson (1984) reported that most hyperthyroid cats have little or no increase in basal serum T4 concentrations following exogenous TSH administration. They suggested that this test may be of value in the diagnosis of feline hyperthyroidism when basal thyroid hormone concentrations are borderline or only slightly elevated. These reports are discussed in more detail in Section 13.9.

Other biochemical changes. Although some early case reports and reviews of feline hyperthyroidism stated that serum biochemical tests were of no diagnostic value (Peterson, Johnson and Andrews, 1979; Peterson, Becker and Hurley, 1980; Peterson et al., 1981; Fox, 1983; Hoenig, 1983), most authors report changes in many biochemical parameters in the disease.

The most commonly found abnormalities are mild to moderate elevations in one or more of the following enzymes: serum alkaline phosphatase (AP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (Holzworth et al., 1980; Kruth, 1980; Theran and Holzworth, 1980; Jones and Johnstone, 1981; McMillan and Scherding, 1981; Hoenig et al., 1982; Olsen, 1982; Fox, Tilley and Liu, 1983; Martin and Capen, 1983; Noxon et al., 1983; Peterson and Yoshioka, 1983; Rochlitz, 1983; Harari, MacCoy, Johnson and Tranquilli, 1984; Cowell and Cowell, 1985). Peterson

(1982, 1983, 1984) stated that such changes were seen in between 50 per cent and 75 per cent of cases. Peterson et al., (1983b) reported that 127 of 131 cases of feline hyperthyroidism had elevations of at least one of AP, ALT, AST or lactate dehydrogenase (LDH).

Mild to moderate azotaemia was found in "approximately" 25 per cent of 131 hyperthyroid cats (Peterson et al., 1983b). However, while other authors have reported elevations of blood urea nitrogen in single cases (Noxon et al., 1983; Harari et al., 1984), Holzworth et al. (1980) failed to find azotaemia in any of ten cases and Hoenig et al. (1982) found an elevated serum creatinine concentration in only one of 24 affected cats.

Mild to moderate hyperphosphataemia was seen in "approximately" 20 per cent of hyperthyroid cats in the absence of renal insufficiency (Peterson, 1982, 1984).

The serum cholesterol concentration of hyperthyroid cats is rarely subnormal. Holzworth et al. (1980) reported this change in only two of ten and Peterson et al. (1983b) in none of 131 cases.

Haematological changes. A number of authors have reported that routine haematological examinations are of no diagnostic value in feline hyperthyroidism (Peterson, Johnson and Andrews, 1979; Kruth, 1980; Peterson, Becker and Hurley, 1980; Jones and Johnstone, 1981; McMillan and Scherding, 1981; Peterson et al., 1981; Webb and Atwell, 1981; Fox, 1983; Hoenig, 1983).

Peterson (1982) reported that mature leukocytosis and eosinopenia are common in feline hyperthyroidism and Cowell and Cowell (1985) made this observation in a single case report.

Peterson et al. (1983b) carried out complete blood counts in 131 cases of feline hyperthyroidism. The PCV and the mean corpuscular volume were increased in 45 per cent of cases. In 14 per cent, the RBC count and the haemoglobin concentration were also increased. The finding of macrocytosis explained why some cats had high PCVs but normal RBC counts and haemoglobin concentrations. A "small number" of cats, however, developed mild anaemia in association with the hyperthyroid state.

Peterson (1983, 1984) presented reviews of these findings.

Urine analysis. Examination of urine in feline hyperthyroidism is non-contributory (Peterson, Johnson and Andrews, 1979; Kruth, 1980; Peterson, Becker and Hurley, 1980; Peterson et al., 1981; Webb and Atwell, 1981; Hoenig, 1983; Noxon et al., 1983). Proteinuria and haematuria were described in two separate case reports (Jones and Johnstone, 1981; Watson, Church, Middleton and Rothwell, 1981), and Peterson et al. (1983b) reported that three cats had glycosuria in association with elevated serum glucose concentrations.

Hyperthyroid cats are able to concentrate urine (Cotter, 1983). Impaired kidney function, as evidenced by low urine specific gravity (less than 1.015) was found in only one of a series of ten (Holzworth et al., 1980) and two of a series of 57 (Peterson et al., 1983b) cases. Such low figures are surprising for groups of aged cats.

Faeces examination. Despite the clinical observation of steatorrhoea in some hyperthyroid cats, Peterson, Johnson and Andrews (1979) reported that faecal fat analyses were normal in five cats. Subsequently, however, Peterson, Becker and Hurley (1980) and

Peterson et al. (1981) reported that 48-hour faecal fat analysis of 28 and 16 cases respectively, revealed marked steatorrhoea with a range of 5.5 to 35.3 g fat (normal is less than 3.5 g). Watson et al. (1981) found split but not unsplit fat in two of three faecal samples from a single hyperthyroid cat.

Peterson et al. (1983b) described more detailed findings on the faecal fat content of five cases of feline hyperthyroidism. The mean daily faecal fat excretion was significantly ($P < 0.01$) greater than normal with a daily excretion of two to 15 times the upper limit of normal. The mean percentage fat digestibility (calculated as the difference between the amount of fat ingested and excreted, divided by the amount ingested) was reduced significantly from normal ($P < 0.01$). The serum T4 concentrations of these animals correlated significantly with the weight of fat excreted and the percentage fat digestibility ($P < 0.05$ in each case).

Peterson (1984) has also reviewed his reports of steatorrhoea in hyperthyroid cats.

Radionuclide studies

Thyroidal radioiodine uptake. Most hyperthyroid cats have increased thyroid RIU when determined 24 hours after administration of the radionuclide (Peterson, 1984).

Peterson, Johnson and Andrews (1979) found the range of thyroid RIU to be 32 per cent to 58 per cent, 24 hours after the administration of an unspecified radioisotope by an unspecified route. Normal values were not cited. Peterson, Becker and Hurley (1980), Peterson et al. (1981), Peterson et al. (1982), Peterson and Becker (1983), Peterson et al. (1983b) and Peterson (1984) found the range in hyperthyroid cats to be 19 per cent to 58 per

cent in 28, 16, 31, 35, 32 and 51 cases respectively, 24 hours after the intravenous administration of 25 uCi of ^{131}I . The mean value in 32 cases was 39.1 ± 2.1 (sd) which was significantly higher ($P < 0.001$) than the mean value in healthy cats (Peterson et al. 1983b).

Hoenig et al. (1982), using 100 uCi of ^{123}I by mouth, measured the thyroidal RIU at six hours in ten and at 24 hours in eight hyperthyroid cats. The mean uptake at six hours was 54.2 per cent (range 11 per cent to 81 per cent) and at 24 hours was 57.8 per cent (range 40 per cent to 97 per cent).

The details of the procedures and the reference ranges for the above reports have been previously discussed in Section 3.8 and summarised in Table 3.7.

Because many factors, including high dietary iodine intake, renal disease and numerous drugs, may decrease thyroidal RIU, such measurements can be a relatively insensitive test for hyperthyroidism in man (McKenzie, Zakarija and Bonnyns, 1979; Ingbar and Woeber, 1981). Thyroidal RIU is, therefore, most useful in determining the ^{131}I dose for the treatment of hyperthyroidism and for research studies. For the former purpose, Turrel et al. (1984) measured the thyroidal RIU in eight hyperthyroid cats at 12-hourly intervals over three to five days, after the intravenous administration of 100 uCi to 300 uCi of ^{131}I . The mean peak uptake was 24.3 ± 9.2 per cent (sd) with a range of 10.0 per cent to 37.9 per cent. Normal values were not reported.

Thyroidal kinetic studies. Peterson, Becker and Hurley (1980) and Peterson et al. (1981) reported a rapid turnover of ^{131}I in five of 16 and four of six hyperthyroid cats studied. No further details were presented.

According to thyroidal kinetic studies of 30 cases, Peterson and Becker (1983) defined two groups of hyperthyroid cats. Thirteen animals with a rapid turnover of ^{131}I (mean 49.8 hours⁻¹) had earlier (six compared with 24 hours) and higher (56.8 per cent compared with 34.2 per cent) mean peak thyroidal RIU relative to 17 cats with slower ^{131}I turnover (mean 148.1 hours⁻¹). The mean T4 and T3 concentrations in the rapid turnover group were 324.3 nmol/l and 7.76 nmol/l respectively. These compared with mean T4 and T3 concentrations of 135.1 nmol/l and 3.39 nmol/l respectively for the slower turnover group. These values were significantly different ($P < 0.002$ in each case).

Peterson, Becker and Hurley (1980) studied the disposition kinetics of an unspecified dose of ^{131}I in four hyperthyroid cats. Urinary excretion predominated over faecal loss, but approximately 25 per cent of administered ^{131}I remained unaccounted for, suggesting either vapour loss or a slow releasing compartment.

Thyroid imaging. In feline hyperthyroidism, thyroid imaging is a very useful procedure to determine unilateral or bilateral lobe involvement, alterations in position of the thyroid lobes, the site of hyperfunctioning accessory/ectopic thyroid tissue and the presence of regional or distant metastasis from a functioning thyroid carcinoma.

Radioactive iodine (^{131}I and ^{123}I) and technetium -99m as pertechnetate ($^{99\text{m}}\text{TcO}_4$) have been used for thyroid imaging in hyperthyroid cats. In a comparison between scans produced with $^{99\text{m}}\text{TcO}_4$ and ^{131}I in hyperthyroid cats, the radionuclide images with both were similar. However, the quality of the

$^{99m}\text{TcO}_4$ scans was usually better than that of the ^{131}I scans (Peterson and Becker, 1984). Unless otherwise stated, the techniques used in the following contributions have been discussed in Section 3.8.

The earliest reports of thyroid imaging of hyperthyroid cats used radioiodine and unilateral lobe involvement was most frequently diagnosed. Thus, Peterson, Johnson and Andrews (1979), Keene and Peterson (1980), Peterson, Becker and Hurley (1980) and Peterson et al. (1981) reported unilateral involvement in 100 per cent of five cases, 1 case only, 71 per cent of 28 cases and 94 per cent of 16 cases respectively. Thyroid uptake varied from a discreet nodule of radionuclide (Peterson, Johnson and Andrews, 1979) to diffuse uptake throughout the lobe(s) (Peterson, Becker and Hurley, 1980). The reasons for the high incidence of unilateral involvement in these early studies compared with a lower incidence in later studies are unclear. Certainly, it was not a result of using radioiodine rather than technetium, as when Peterson and Becker (1984) used each in 32 cases of feline hyperthyroidism, they found 100 per cent agreement between the scans obtained with the two radioisotopes.

Hoenig et al. (1982) first reported the use of $^{99m}\text{TcO}_4$ in thyroid imaging of six hyperthyroid cats. The animals were premedicated with atropine and sedated with ketamine. Subsequently, 1 mCi to 2 mCi of the radioisotope were administered intravenously and the animals scanned with a gamma camera from the cricoid arch to the heart base 30 minutes later. No difficulty was encountered in recognising functional thyroid tissue.

Peterson et al. (1982) carried out scintiscan evaluations of 45 hyperthyroid cats. In all cases, there was evidence of increased

radionuclide uptake in one or both thyroid lobes consistent with the presence of hyperfunctional tumours. In a study of 11 hyperthyroid cats using the same technique, Peterson et al. (1983a) found bilateral thyroid lobe enlargement in nine (82 per cent) and unilateral lobe enlargement in two (18 per cent).

Peterson et al. (1983b) used $^{99m}\text{TcO}_4$ for thyroid imaging of 126 hyperthyroid cats. Enlargement and increased radionuclide accumulation was found unilaterally in 36 cases (29 per cent) and bilaterally in 90 cases (71 per cent). In all cats with unilateral involvement, the contralateral lobe was suppressed completely and could not be visualised. The left lobe was affected in 21 cases (58 per cent) and the right in 15 cases (42 per cent). Where there was bilateral involvement, both lobes were of similar size in 31 cases (34 per cent) and of unequal size in 59 (66 per cent). In 16 cats (13 per cent), there was no palpable goitre and in these, scans revealed that the affected lobes had descended into the thoracic cavity. Thyroid imaging showed extension of neck swellings into the thoracic cavity in two cats (1.6 per cent). Biopsies from these subsequently confirmed adenocarcinoma formation. This study was subsequently extended to 135 cases (Peterson and Becker, 1984). They commented that although radionuclide uptake was fairly uniform in distribution in all cats with unilateral lobe involvement, heterogeneous accumulation of radioisotope occurred in 27 per cent of cases with bilateral involvement. Summaries of these studies were also published by Peterson (1982, 1983, 1984).

Peterson and Yoshioka (1983) used thyroid imaging to identify the site of hyperfunctional thyroid tissue which was not palpable on physical examination of a single hyperthyroid cat. After

premedication with atropine and tranquillising with ketamine, 1.8 mCi $^{99}\text{TcO}_4$ was administered intravenously. Ten minutes later, with the patient in dorsal recumbency, 150,000 gamma camera scintiplates were obtained using a low energy, all-purpose collimator. Three small foci on the right side of the neck and one large intense focus of activity on the left side of the neck were identified.

Turrel et al. (1984) performed thyroid imaging on 11 cats, some of which had been previously treated unsuccessfully for hyperthyroidism by surgery (two cases) and propylthiouracil (seven cases), prior to subsequent thyroid ablation by ^{131}I . Images were obtained on a large field of view gamma camera utilising a converging collimator, 20 minutes after the intravenous injection of "approximately" 1 mCi $^{99\text{m}}\text{TcO}_4$.

Rectilinear scanning has also been employed for thyroid imaging in hyperthyroid cats. Hoenig et al. (1982) carried out the technique using ^{123}I as described in Section 3.8 in 18 animals. Resolution by this technique was usually inadequate to permit differentiation between unilateral and bilateral involvement of the thyroid. A summary of this work was published by Hoenig (1983).

Noxon et al. (1983) used rectilinear scanning to identify the site of hyperfunctional "ectopic" thyroid tissue at the thoracic inlet of a 13-year-old, domestic short-haired cat. 5.7 mCi $^{99\text{m}}\text{Tc}$ pertechnetate was administered intravenously and, three hours later, the cat was anaesthetised with halothane, placed in dorsal recumbency and imaging carried out with a Picker Magna V rectilinear scanner. The procedure contributed to successful surgical removal of the thyroid mass. It is possible that the thyroid tissue

identified was not truly ectopic but a hyperfunctional lobe which had subsequently moved from its original site through the thoracic inlet.

The main value of thyroid imaging in a cat with confirmed hyperthyroidism is to indicate whether one or both lobes are abnormal and thus, whether unilateral or bilateral thyroidectomy is required. Where these facilities are not available, the clinician can only make this decision on gross appearance of the lobe at time of surgery or as a result of thyroid biopsy.

Thyroid biopsy. Thyroid biopsy may be used to confirm that a cervical mass is of thyroidal origin and to identify the specific histological abnormality. In hyperthyroid cats, the technique is most frequently used to sample a lobe which is equivocally abnormal in size, colour or location at the time of unilateral thyroidectomy, thus indicating whether further surgery may be required. Because biopsy results may demonstrate absence of pathological change, the procedure may prevent the lifelong administration of thyroid hormone replacement therapy or reduce the incidence of hypoparathyroidism (Black and Peterson, 1983).

Thyroid biopsy may be carried out by closed or open techniques but, for the reasons outlined above, the former is rarely indicated in cats. Black and Peterson (1983) described two methods of carrying out open thyroid biopsies in cats. In both, the surgical approach to the gland is as described subsequently, and the biopsy specimen is taken from the caudal aspect of the gland which is the least vascular and which ensures preservation of the external parathyroid.

One technique involves using a suture of polyglycolic acid or silk to amputate the caudal tip of the gland after ligation of the caudal vasculature. If this is unsuccessful a wedge biopsy of the same area can be performed, controlling haemorrhage with digital pressure or with a deep, horizontal mattress suture.

Post-mortem examination. The results of a small number of post-mortem examinations of hyperthyroid cats have been published.

Jones and Johnstone (1981) commented on the emaciated appearance of one case. Liu, Peterson and Fox (1984) reported that the mean bodyweight of 23 cases examined post-mortem was significantly ($P < 0.001$) less than that of clinically normal cats.

Hypertrophic cardiomyopathy is commonly found at post-mortem examination of hyperthyroid cats. Harari et al. (1984) examined a cat which was euthanased because of a recurrent peripheral arteriovenous fistula at the left elbow, an infiltrative sublingual squamous cell carcinoma and hyperthyroidism. The heart was globoid, the apex rounded and the left ventricular wall was moderately thickened.

Liu, Peterson and Fox (1984) found hypertrophic cardiomyopathy in 100 per cent of 23 hyperthyroid cats examined post-mortem. The ratio of heart weight to bodyweight was significantly greater ($P < 0.001$) in these animals than in normal cats and cats with primary cardiomyopathy. Twenty animals (87 per cent) had symmetrical hypertrophy of the ventricular septum and the left ventricular free wall, whereas the remaining three had disproportionate thickening of the ventricular septum compared with the free wall, similar to that which is found in cats with asymmetric hypertrophic cardiomyopathy. Histological cardiac

abnormalities included large hyperchromatic nuclei, interstitial fibrosis, endocardial fibroplasia, fibrosis of the atrioventricular node and marked disorganisation of cardiac muscle cells. Myocardial fibrosis was also reported in a single case by Watson et al. (1981).

Cowell and Cowell (1985) reported a single case of feline hyperthyroidism which was associated with a pseudochyloous thoracic effusion. The heart showed mild degenerative changes and it was postulated that these were secondary to hyperthyroidism, with the cardiomyopathy precipitating the effusion.

Hepatic changes in feline hyperthyroidism include marked hyperaemia (Cowell and Cowell, 1985), diffuse atrophy of hepatic cell plates (Jones and Johnstone, 1981), fatty change (Jones and Johnstone, 1981; Liu, Peterson and Fox, 1984), bile pigment accumulation (Jones and Johnstone, 1981) and partial fibrosis (Watson et al., 1981).

Thirteen of 23 cats examined showed considerable engorgement and tortuosity of alveolar capillaries (Liu, Fox and Peterson, 1984). Transmural thickening of the pulmonary arteries, multifocal areas of atelectasis and occasional pleural thickenings were observed in a single case (Cowell and Cowell, 1985).

Liu, Fox and Peterson (1984) reported that eight of 23 hyperthyroid cats showed nodular hyperplasia of the zonae glomerulosa and fasciculata of the adrenal glands. Three cases had diffuse hyperplasia of cells of the zona glomerulosa.

Jones and Johnstone (1981) reported dermatohistopathology consisting of mild hyperkeratosis, severe atrophy of adnexal structures and hypertrophy of piloerector muscles in a single case of feline hyperthyroidism.

Mild to extensive amyloidosis has been reported in a small number of thyrotoxic cats. Jones and Johnstone (1981) described mild to moderate amyloid deposition in the perivascular connective tissue of lung, lymph nodes, skin, adrenals, thyroid, liver and spleen of a single case. Watson et al. (1981) found extensive amyloid deposition in the glomeruli and between the medullary tubules, and in the adrenal and thyroid glands of a hyperthyroid cat. In both of these reports, it was suggested that the amyloid may have been a coincidental finding. However, localised thyroid amyloidosis has been reported previously in association with feline thyroid tumours (Lucke, 1964; Leav et al., 1976).

Treatment. Spontaneous remission of feline hyperthyroidism does not occur (Peterson, 1984). The disease may be treated in three ways: by surgery, with radioactive iodine (^{131}I) or by long-term use of an antithyroid drug.

The treatment adopted will depend on several factors including the presence or absence of major medical disorders, the availability of an experienced surgeon or nuclear medicine department and the wishes of the owner (Peterson, 1984).

Surgery. Surgical thyroidectomy is the treatment of choice for most thyrotoxic cats (Black and Peterson, 1983; Birchard, Peterson and Jacobson, 1984). The surgical procedure is relatively simple (Peterson, 1984) and safe (Peterson, Birchard and Mehlhaff, 1984). However, because hyperthyroidism affects many organ systems, the anaesthetic and surgical risks in affected cats are increased (Peterson et al., 1983b). While some authors (e.g. Theran and Holzworth, 1980) felt that pre-operative treatment of hyperthyroid cats did not affect the outcome of surgery, Birchard, Peterson and

Jacobson (1984) reported that six of eight cats which died during or immediately after thyroidectomy had not received such treatment.

Pre-operative treatment. Antithyroid drugs, and potassium iodine alone or in combination with propranolol have been used for pre-operative treatment of hyperthyroid cats.

Peterson (1981) felt that antithyroid drugs were the method of choice for this procedure. The two most widely used drugs are the thiocarbamide derivatives propylthiouracil (PTU) and methimazole (MMI). They act mainly by blocking incorporation of iodine into the tyrosyl groups in thyroglobulin and by preventing the coupling of MIT and DIT (Green, 1978). In addition, PTU inhibits conversion of T4 and T3 in peripheral tissue (Larsen, 1978).

Recommended initial daily doses of PTU and MMI are 150 mg and 15 mg per cat respectively. Although single daily doses have been reported to be adequate in some cats, three times daily administration appears to provide a more reliable response (Peterson, 1984).

The length of time needed for either drug to return T4 and T3 concentrations to normal is determined by the initial degree of hyperthyroidism, the amount of thyroid hormones stored in the gland and the extent to which thyroid hormone synthesis is inhibited (Peterson, 1984). Most hyperthyroid cats treated with the above regime become euthyroid within one to three weeks (Black and Peterson, 1983; Hoenig, 1983), or two to three weeks (Peterson, 1981), although Bradley and Feldman (1982) reported that five of 13 cases continued to have elevated serum thyroid hormone concentrations after 21 days' treatment with PTU by this regime. Dosages of 200 mg to 300 mg PTU per day have been used if there is no decrease in

serum thyroid hormone concentrations by this time (Peterson, 1981, 1982).

Side effects of antithyroid drug therapy are common (Peterson, 1981, 1982) and occur more frequently with PTU than MMI. These reactions are usually mild but serious immune-mediated drug reactions have also been recorded (Peterson, Cavanagh and Hurvitz, 1983; Peterson, Hurvitz, Lieb, Cavanagh and Dutton, 1984) and are described subsequently.

Propranolol, a beta-adrenergic receptor antagonist, does not lower elevated serum thyroid hormone concentrations but prevents many of the cardiovascular and neuromuscular effects of excess thyroid hormones and controls the tachycardia and hyperexcitability associated with feline hyperthyroidism (Peterson, 1982; Hoenig, 1983; Peterson, 1983; Birchard, Peterson and Jacobson, 1984). Commonly recommended dosages for cats are 2.5 mg three times daily (Black and Peterson, 1983) or 2.5 to 5.0 mg three times daily (Peterson, 1982, 1983; Birchard, Peterson and Jacobson, 1984; Peterson, 1984) for seven to 14 days before surgery. Birchard, Peterson and Jacobson (1984) and Peterson, Birchard and Mehlhaff (1984) used propranolol alone as pre-operative treatment for feline hyperthyroidism but it has also been combined with potassium iodide therapy.

Large doses of stable iodine are reported to block T₄ and T₃ release from the human thyroid gland and to lower serum thyroid hormone concentrations (McKenzie, Zakarija and Bonnyns, 1979; Ingbar and Woeber, 1981). Thus, Hoenig (1983) recommended three to five drops of saturated potassium iodide solution daily to render cats euthyroid prior to thyroidectomy and to prevent complications such

as cardiac arrhythmias during surgery, or post-operative "thyroid storm" (hyperthermia, disorientation, disturbed cardiac function). However, iodine alone is reported to have major limitations as antithyroid therapy. Serum T4 and T3 concentrations may never return to the euthyroid range and the drug may lose its antithyroid effect within a few weeks (Peterson, 1982). Thus, the combined use of potassium iodide and propranolol has been recommended as being equally effective as PTU or MMI for the pre-surgical treatment of feline hyperthyroidism (Black and Peterson, 1983), or as an alternative in affected cats that cannot tolerate PTU (Peterson, 1981, 1983, 1984). Recommended doses of potassium iodide, when used together with propranolol, in hyperthyroid cats, have been 50 mg to 100 mg daily (Peterson, 1982, 1983, 1984) and two to three drops of the saturated solution daily for seven to 14 days before surgery (Black and Peterson, 1983). Potassium iodide is said to have a brassy taste and to cause excessive salivation and partial to complete anorexia in some cats (Black and Peterson, 1983).

Anaesthesia and management during surgery. Anaesthesia of the hyperthyroid cat requires a regime which minimises the risk of death from cardiac arrhythmia. Premedication with acepromazine, administered intramuscularly, has proved effective as it reduces the autonomic manifestations of hyperthyroidism and causes sedation (Black and Peterson, 1983; Birchard, Peterson and Jacobson, 1984). Anticholinergic agents such as atropine are not usually used because they cause sinus tachycardia and are known to enhance anaesthetic-induced cardiac arrhythmias (Black and Peterson, 1983). If required, glycopyrrolate is the anticholinergic of choice (Peterson, Birchard and Mehlhaff, 1984) since it has minimal effects on cardiac rate and

rhythm (Short and Miller, 1978). Thiamylal sodium or ketamine are suitable agents for induction of anaesthesia (Birchard, Peterson and Jacobson, 1984). Nitrous oxide alone (Black and Peterson, 1983), or in combination with halothane or methoxyflurane (Peterson, Birchard and Mehlhaff, 1984) may be used to maintain anaesthesia.

Nine of a series of 85 hyperthyroid cats developed atrial or ventricular arrhythmias during surgery. These were successfully controlled using 0.1 mg to 0.2 mg intravenous propranolol (Birchard, Peterson and Jacobson, 1984).

Surgical technique. The surgical approach to the thyroid gland has been described by Black and Peterson (1983), Birchard, Peterson and Jacobson (1984) and Peterson, Birchard and Mehlhaff (1984). The cat is positioned in dorsal recumbency with the forelimbs pulled caudally and the neck slightly hyperextended. A ventral, midline skin incision is made from the larynx to the manubrium. The sternohyoideus and sternothyroideus muscles are then separated by blunt dissection in the midline and retracted to reveal the thyroid lobes.

In the case of unilateral lobe pathology, Black and Peterson (1983) recommended unilateral extracapsular thyroidectomy and parathyroidectomy. The cranial and caudal thyroid vessels are ligated and transected and dissection of the loose cervical fascia surrounding the gland allows its removal. However, subsequently, Birchard, Peterson and Jacobson (1984) and Peterson, Birchard and Mehlhaff (1984) recommended that intracapsular thyroidectomy should be carried out so as to preserve both external parathyroid glands, in cases of both unilateral and bilateral thyroid enlargement. The internal parathyroids are embedded in the substance of the thyroid and cannot be preserved (see Section 3.3).

To carry out intracapsular thyroidectomy, the caudal thyroid vein is ligated and transected. A nick incision is then made in an avascular area of the thyroid capsule on the caudal aspect of the gland. This incision is then extended cranially with scissors until the entire capsule is opened. The thyroid parenchyma is then bluntly removed from the capsule using sterile cotton-buds leaving the thyroid capsule and the external parathyroid gland intact. The cranial thyroid vessels should not be disturbed, because transient or permanent ischaemia of the parathyroid gland may result. If thyroid tissue fragments during separation from the capsule, all pieces must be removed even if portions of capsule are removed with them. If there is bilateral lobe involvement, the procedure is repeated for the opposite lobe.

In man, even if the parathyroid glands are successfully preserved, their function may be impaired by oedema and scarring (Freeman, 1970). The incidence of permanent hypoparathyroidism was decreased dramatically with routine autotransplantation into the forearm musculature, of any parathyroid tissue which became devascularised (Paloyan, Lawrence and Paloyan, 1977). Thus, Peterson, Birchard and Mehlhaff (1984) have recommended the same procedure in cats. However, Theran and Holzworth (1980) reported that accessory parathyroid tissue is usually present in sufficient amounts in cats to maintain function or to restore it within a few days, even after total parathyroidectomy.

The surgical field is carefully examined for haemostasis before closing the incision routinely.

Post-operative complications. Reported post-operative complications include hypoparathyroidism, Horner's syndrome and voice changes.

Hypoparathyroidism may occur after the parathyroid glands are injured, devascularised or inadvertently removed in the course of surgery (Peterson, 1982, 1983). This results in hypocalcaemia which may be manifested as heightened neuromuscular irritability with muscle tremors, tetany and generalised convulsions. As only one (Peterson, 1984) or one to two (Birchard, Peterson and Jacobson, 1984) parathyroid glands are required to maintain normocalcaemia, hypoparathyroidism only occurs as a sequel to bilateral thyroidectomy.

Hoenig et al. (1982) reported that six of 19 hyperthyroid cats treated by bilateral thyroidectomy became hypocalcaemic despite preserving at least one parathyroid in all but two cases. In four, the hypocalcaemia was evident within eight hours of surgery but not until four to five days later in the remainder. Birchard, Peterson and Jacobson (1984) reported that eight of 53 hyperthyroid cats (15 per cent) treated by bilateral thyroidectomy had low serum calcium concentrations when tested one to four days after surgery. However, only four animals showed signs and were treated. Peterson (1982, 1983) also reported that it is not necessary to treat hypocalcaemia in the absence of clinical signs. However, Martin and Capen (1983) stated that treatment should be given routinely to any cat in which the serum calcium concentration falls below 2 mmol/l. Because of the possibility of post-surgical hypoparathyroidism, serum calcium determinations should be carried out within eight hours of bilateral thyroidectomy (Hoenig, 1983), daily for at least two days following surgery (Peterson, 1982, 1983), and daily until it has stabilised within the normal range (Peterson, 1984).

Treatment of hypoparathyroidism involves the use of calcium with or without vitamin D. Black and Peterson (1983) recommended beginning treatment of the acute stage with 1.5 ml/kg of ten per cent calcium gluconate by slow intravenous injection. Subsequently, calcium may be administered orally. Hoenig et al. (1982) reported that the daily dose of calcium gluconate required to maintain normocalcaemia in affected cats post-surgery was 0.6g to 4g and that there was no correlation with the initial serum calcium concentration.

Vitamin D may be administered together with calcium as soon as signs of hypocalcaemia are detected (Holzworth et al., 1980; Theran and Holzworth, 1980; Hoenig et al., 1982; Martin and Capen, 1983) or used if oral calcium alone is not sufficient to maintain normal serum calcium concentrations (Black and Peterson, 1983). However, vitamin D therapy must be used cautiously in cats as overdosing may result in hypercalcaemia and secondary renal damage (Theran and Holzworth, 1980).

Unspecified dosages of vitamin D₂ (Holzworth et al., 1980), and 50,000 iu D₃ (Hoenig et al., 1982) and loading dosages of 0.6 g to 0.8 g with subsequent daily maintenance dosages of 0.2 g of dihydrotachysterol (Hoenig et al., 1982) have been recommended for cats. Hoenig (1983) commented that dihydrotachysterol has the advantage over other vitamin D analogues because it does not require parathyroid hormone-mediated activation by the kidney. It is activated by the liver and is non-cumulative (Harrison, Lifshitz and Blizzard, 1967).

In most cases, iatrogenic hypocalcaemia in cats resolves spontaneously, weeks to months after surgery (Peterson, 1982, 1983).

Birchard, Peterson and Jacobson (1984) reported that treatment of all four of their cases was discontinued after two to six months without recurrence of hypocalcaemia. In such cases, the hypocalcaemia probably results from reversible parathyroid damage and ischaemia incurred during surgery, or accessory parathyroid tissue may become functional (Peterson, 1984).

Horner's syndrome may occur as a sequel to thyroidectomy in cats (Peterson, 1982, 1983) and arises due to injury to the cervical sympathetic trunk (Hoenig, 1983). One of 85 cats treated by Birchard, Peterson and Jacobson (1984) developed Horner's syndrome.

Damage to the recurrent laryngeal nerves may occur during thyroidectomy in cats because they lie close to the thyroid lobes and are less than 1 mm in diameter. Resultant signs include altered voice, cough and harsh respiratory sounds (Olsen, 1982). Holzworth et al. (1980) and Birchard, Peterson and Jacobson (1984) reported that one of ten and one of 85 hyperthyroid cats respectively, developed such changes after surgical treatment.

Long-term management. Following thyroidectomy for hyperthyroidism, serum thyroid hormone concentrations become normal or subnormal within 24 to 48 hours (Peterson, Becker and Hurley, 1980; Peterson, 1982, 1983; Birchard, Peterson and Jacobson, 1984). After hemithyroidectomy for unilateral lobe involvement, T4 and T3 concentrations are usually subnormal for six weeks (Hoenig, 1983) or two to three months post-operatively (Peterson, 1982; Black and Peterson, 1983; Peterson, 1983) but T4 supplementation is rarely required during this period (Theran and Holzworth, 1980; Peterson, 1984).

Some authors state that bilateral thyroidectomy in hyperthyroid cats results in permanent hypothyroidism (Theran and Holzworth, 1980; Olsen, 1982; Martin and Capen, 1983). Other workers have reported that serum T4 and T3 concentrations in such cases return to normal within six weeks (Hoenig et al., 1982) or three to six months (Birchard, Peterson and Jacobson, 1984) of surgery. The source of thyroid hormones after bilateral thyroidectomy may be ectopic thyroid tissue (Hoenig et al., 1982) or regeneration of small remnants of thyroid tissue which remain attached to the capsule (Peterson, 1984). Most authors recommend beginning T4 supplementation within 24 to 48 hours of surgery. Subsequently, T4 administration may be for life (Theran and Holzworth, 1980; Martin and Capen, 1983) or simply until endogenous hormone is once again produced at normal concentrations (Peterson, 1984). Replacement dosages of thyroid hormones for hypothyroid cats are reviewed in Section 3.12.

After successful surgery, the signs of hyperthyroidism rapidly disappear. Hoenig et al. (1982) described marked clinical improvement with weight gain and evidence of regrowth of hair within two weeks and Peterson et al. (1982) reported that ECG abnormalities and associated cardiovascular signs resolved in most cases.

Peterson (1982) commented that relapse rarely, if ever, occurred after thyroidectomy for hyperthyroidism when the condition was due to unilateral lobe changes. Birchard, Peterson and Jacobson (1984) reported that four of 53 hyperthyroid cats developed recurrence of hyperthyroidism within eight to 44 months of bilateral thyroidectomy. They felt that this was due to regrowth of adenomatous thyroid tissue attached to the thyroid capsule.

Recurrence may also result from failure to remove totally thyroidal adenocarcinomas (Hoenig et al., 1982). Thus, Peterson, Birchard and Mehlhaff (1984) recommended that all hyperthyroid cats treated by surgical thyroidectomy should have their serum thyroid hormone concentrations monitored at six to 12 month intervals to ensure that relapse does not occur.

Radioactive iodine. In human medicine, radioactive iodine (^{131}I) provides a simple, effective and safe treatment for hyperthyroidism (Peterson et al., 1983a). Radioactive iodine is concentrated within the thyroid gland and selectively irradiates and destroys functioning thyroid tissue (McKenzie, Zakarija and Bonnyns, 1979; Ingbar and Woeber, 1981). Dosages should, however, be carefully calculated as persistent or recurrent hyperthyroidism may result from inadequate therapy and hypothyroidism results from overdosage. ^{131}I has also been used to treat feline hyperthyroidism.

In an early contribution, Peterson, Becker and Hurley (1980) commented that they had successfully treated one hyperthyroid cat with ^{131}I . No further details were given.

Peterson and Becker (1983) gave brief details of 15 cases which were treated with between 1 mCi and 5 mCi ^{131}I . Serum T4 and T3 concentrations returned to the respective reference ranges within two to nine days with a mean of 4.1 days. Hypothyroidism developed in two cats while two relapsed needing retreatment.

Both Peterson et al. (1983a) and Turrel et al. (1984) attempted to individualise doses of ^{131}I by carrying out radioactive iodine tracer studies to determine peak RIU and biological half-lives. Subsequently, the administered dose was

calculated on the basis of these results and the estimated thyroid gland weight.

Peterson et al. (1983a) attempted to deliver 15,000 rad to the thyroid gland in each of 11 cases. The serum T4 concentration decreased to the normal range in one to nine days with a mean of 4.1 ± 0.7 days. No serious side-effects were noted. Signs consistent with hypothyroidism developed in one cat and its serum T4 concentration was found to be subnormal, requiring thyroid hormone supplementation. One cat relapsed after 18 months but responded to further ^{131}I therapy. The remaining nine cats remained euthyroid in follow-up periods of one to 26 months.

Turrel et al. (1984) treated 11 hyperthyroid cats with ^{131}I , seven of which had been previously treated unsuccessfully with PTU and two by hemithyroidectomy. Their treatment goal was to deliver 20,000 rad to hyperactive thyroid tissue using doses of 1.0 mCi to 5.9 mCi ^{131}I . However, retrospective calculations showed that radiation doses actually ranged from 7,100 rad to 64,900 rad. Adverse effects of ^{131}I therapy such as radiation thyroiditis or "thyroid storm" were not observed. A transient change in voice was noted in one cat. Six hyperthyroid cats became euthyroid after a single treatment while one required retreating after six months. Clinical improvement was noticeable and serum T4 concentrations were in the reference range within one month of therapy. Two cats had partial responses to ^{131}I therapy but remained clinically and biochemically hyperthyroid. Two cats became hypothyroid. The relationship between the theoretically required ^{131}I dose and the ultimate thyroid status appeared to be affected by: (i) the initial T4 concentrations, with animals having high concentrations

responding less well, (ii) previous PTU therapy, there being some evidence of this drug increasing radioresistance, (iii) the size of the gland, with larger glands responding less well.

The use of radioactive iodine to treat feline hyperthyroidism has also been reviewed by Peterson (1982, 1983, 1984).

While radioactive iodine therapy is an effective treatment for feline hyperthyroidism, the lack of suitable facilities for this type of treatment in animals severely limits its usefulness in veterinary practice.

Long-term antithyroid drug therapy. This therapeutic approach has been suggested for the management of feline hyperthyroidism where unrelated medical conditions increase the surgical risk, owners refuse surgical treatment and ^{131}I therapy is unavailable (Peterson, 1981, 1982). Both PTU and MMI have been used in hyperthyroid cats but no detailed studies of results with the latter drug have been published. Unless otherwise specified, the following comments refer to PTU therapy.

As discussed previously with respect to pre-operative treatment, most workers report that initial daily doses of 150 mg PTU or 15 mg MMI will reduce the serum T4 and T3 concentrations in most hyperthyroid cats to the normal range within one to three weeks. However, there are a number of disadvantages to long-term antithyroid drug therapy, in particular with regard to maintenance of the euthyroid state and the development of drug side-effects.

Because these drugs do not remove the underlying thyroid lesion, if they are discontinued or given irregularly (as may result from poor owner compliance), the serum thyroid hormone concentrations increase again (Peterson, 1982, 1984). It may also

be difficult to maintain the euthyroid state without regular changes in the dosages of the drug. Thus, Peterson (1981) reported that the majority of hyperthyroid cats treated long-term with 150 mg PTU daily developed subnormal thyroid hormone concentrations and showed signs of hypothyroidism. However, in further contributions, the same author (Peterson, 1982, 1983, 1984) commented that relapse into the thyrotoxic state usually occurs in due course if the dosage is reduced below 150 mg PTU daily. In addition, hyperthyroid cats may become "resistant" to the effects of antithyroid drugs and subsequently require an increased dose to maintain normal serum thyroid hormone concentrations.

Holzworth et al. (1980) were the first workers to report the use of PTU in the management of feline hyperthyroidism. At 150 mg PTU daily, four of six cases continued to eat ravenously but stopped losing weight. The remaining two became anorexic immediately and PTU therapy was ceased.

McMillan and Scherding (1981) used 50 mg PTU twice daily to treat a single case of feline hyperthyroidism. The cat died four months after the diagnosis was made, showing difficulty in eating and swallowing.

Bradley and Feldman (1982) treated 13 cats with long-term PTU. Eleven cats subjectively were improved according to their owners and ten cats gained weight.

Minor adverse reactions to PTU therapy in cats are common and include lethargy, vomiting (Peterson, 1981; Hoenig, 1983), decreased appetite, facial swelling and pruritus (Peterson, 1981). However, in an early contribution, Peterson (1981) commented that more serious side-effects (hepatopathy and immune-mediated

haemolytic anaemia) developed in two of 16 hyperthyroid cats treated with PTU and subsequently other complications were noted.

Peterson and Becker (1983) briefly commented on serious drug reactions which occurred in six of 20 hyperthyroid cats (30 per cent) treated solely with PTU. These included anaemia, thrombocytopenia and a lupus-like reaction. Peterson, Cavanagh and Hurvitz (1983) gave further information about these animals and subsequently Peterson et al. (1984) extended the series to report on adverse reactions of PTU therapy in nine of 105 cats.

Clinical signs developed after 19 to 37 days (with a mean of 24.8 days), and included lethargy (nine cats), weakness (seven), anorexia (six), bruising (four), oral haemorrhage (three), aural haemorrhage (two), and haematemesis (one). Physical examination revealed pale mucous membranes (nine cats), petechial and ecchymotic haemorrhages of the skin and oral cavity (seven), fundic haemorrhage (one) and icterus (one). In all nine cats, routine haematological tests showed severe anaemia and thrombocytopenia. Granulocytopenia, the most frequent haematological reaction to PTU therapy in man (Parker, 1980), was not observed but has been recorded in cats taking MMI (Peterson, 1984). A bone marrow biopsy in two cats showed erythroid and megakaryocytic hyperplasia. The direct antiglobulin (Coombs') test was positive in all seven cats evaluated and the serum antinuclear antibody (ANA) titre was greater than 1:10 in five of eight cats tested. All cats were negative for feline leukaemia virus antigen.

Five cats were euthanased. The remaining four responded to cessation of PTU, whole blood transfusions and oral prednisone. Two cats also received cytotoxic therapy (vincristine and

cyclophosphamide). In these, the anaemia and thrombocytopenia resolved and the Coombs' and ANA tests became negative within two weeks.

Cats that have developed a serious toxic reaction to one of the antithyroid drugs should not be treated with the other since cross-sensitivity may occur (Peterson, 1984).

Thus, to maintain normal circulating thyroid hormone concentrations and to monitor for serious adverse reactions to long-term drug therapy, a physical examination, complete blood count and serum T4 and T3 determinations should be carried out, initially at weekly intervals, especially between the second and sixth week of PTU therapy when most serious adverse reactions occur (Peterson et al., 1984). Subsequently, monitoring at monthly intervals is probably satisfactory (Peterson, 1984).

Various clinical aspects of naturally-occurring, confirmed cases of feline hyperthyroidism have also been reviewed by Schaer (1981), Legendre (1983), Moore (1983), Oliver and Greene (1983), Scherding (1983) and Tilley, Liu and Fox (1983).

Experimentally-induced cases of feline hyperthyroidism

Experimental hyperthyroidism has been induced in kittens by dietary means and in adult cats by the administration of thyroid extract or T4.

Roberts and Scott (1961) and Scott, Greaves and Scott (1961) reported that kittens fed on diets with fluctuating iodine content, (such as when a fish diet rich in iodine was replaced for several weeks by a meat diet low in iodine), had thyroid glands which were hyperplastic. They felt that these animals might have been thyrotoxic but on histological and other evidence available, they were not able

to be certain whether this was the case, or whether the thyroid was showing compensatory hypertrophy due to iodine deficiency or the presence of a goitrogen in meat. However, Scott (1966, 1975a), citing this work, reported that these animals were hyperthyroid. The cats were hyperexcitable and very active for brief periods, after which they became exhausted and tachypnoeic. Laboratory studies to confirm the hyperthyroid state were not carried out and it is unlikely that these animals were actually thyrotoxic.

Hyperthyroidism has been induced experimentally in cats by a number of medical research groups. Details of the regimes employed and biochemical changes reported are given in Table 3.12.

Buccino, Spann, Pool, Sonnenblick and Braunwald (1967) induced hyperthyroidism in 13 cats. The serum PBI and cholesterol concentrations of 12 animals studied were significantly elevated and reduced respectively ($P < 0.01$ in each case) but the heart rate was not significantly different, as compared to euthyroid animals. In hyperthyroid cats, the cardiac index, the mean systolic ejection rate and the total oxygen consumption were all significantly elevated above normal. The authors concluded that the thyroid state profoundly affects the intrinsic contractile state of cardiac muscle, primarily by altering the speed of shortening of contractile elements, and that the cardiovascular dynamic changes in hyperthyroidism result in part from the effects of thyroid hormones or something produced in response to thyroid hormones.

Aliev and Gaidina (1973) showed that the administration of excess thyroid hormones to cats changed the modulatory effect of supraspinal structures on spinal reflex activity.

In the course of experimental studies on contractile function of the heart, Skelton and Sonnenblick (1974) induced acute and chronic hyperthyroidism in cats. Short-term administration of T4 did not result in alterations in ventricular size, while prolonged T4 administration resulted in massive biventricular hypertrophy. When T4 administration was stopped, both ventricle sizes regressed to normal within 12 months.

Strauer and Scherpe (1975) reported that cats with experimental thyrotoxicosis showed an increased responsiveness of the ventricular myocardium to the negative inotropic effect of propranolol.

Other studies involving the experimental induction of thyrotoxicosis in cats include those by Sobel, Dempsey and Cooper (1969) and Gol'ber, Gaidina and Ignatkov (1971).

3.12 FELINE HYPOTHYROIDISM

Introduction

Hypothyroidism (Gull's disease) is a multisystemic disorder associated with a deficiency of thyroid hormone activity. Despite numerous anecdotal reports of the condition in cats, a case of naturally-occurring, acquired, feline hypothyroidism has never been definitively diagnosed, although a case of primary hypothyroidism-associated dwarfism in a kitten has been described (Arnold, Opitz, Grosser, Bader and Eigenmann, 1984). This section reviews these reports and those in which hypothyroidism was experimentally or therapeutically induced.

Naturally-occurring cases of feline hypothyroidism

In 1914, Carlson reported that goitre in cats was endemic in the Chicago and Great Lakes region of the USA. The mean ratio of

thyroid weight to bodyweight in six affected queens was 1:6,400 (normal 1:12,000) and in 27 kittens born to them, 1:2,424 (normal 1:4,705). The adult hyperplastic thyroids showed decreased acini and colloid, columnar instead of cuboidal cells and a marked increase in interacinar cells. Thyroid glands from affected kittens were enlarged with retarded formation of acini. The author concluded that "the goitre of the newborn is due to some pathological condition of the maternal blood".

Bourgeois (1933) reported that the "great majority" of cats in Bern had goitre by comparison with animals from Paris or Hanover. The degree of goitre was independent of age and no cause was suggested. It is likely that the goitres reported in these early contributions were due to local iodine deficiency.

Holzworth, Husted and Wind (1955) gave details of an obese, 15-year-old, neutered male, domestic short-haired cat which presented with a left fore-leg lameness. It had sparse, brittle hair over the flanks, atonic muscles and was weak, anxious and restless. There was tachycardia and subsequently tachypnoea and polydipsia. Although thyroid function tests were not carried out, a tentative diagnosis of hypothyroidism was made. At post-mortem examination, bilateral thyroid adenocarcinoma with arterial thrombosis were found. Retrospectively, the authors considered that the cat might have been hyper rather than hypothyroid. The historical and clinical features of anxiety, hyperactivity, tachycardia, tachypnoea and polydipsia suggest that the cat was, indeed, probably hyperthyroid.

Meier and Clark (1958) diagnosed hypothyroidism in five cats, "on the presence or lack of functional disturbances associated with

thyroid changes" but, in apparent contradiction, went on to report that, "In cats, clinical hypothyroidism practically does not exist, there being no symptoms specifically referable to decreased function".

Lucke (1964) studied the thyroid glands obtained from 75 consecutive feline post-mortem examinations. The histological appearance of one case resembled the involutionary stage of iodine deficiency goitre.

Joshua (1965, 1971, 1979) diagnosed hypothyroidism clinically in association with a decrease in quantity and quality of the coat, excessive sluggishness, a reluctance to jump without evidence of a painful focus and a slow pulse rate.

Conroy (1968) reported that hypothyroidism, sometimes associated with widespread diffuse alopecia, occurred infrequently in cats. He cited a plasma cholesterol concentration of greater than 3.9 mmol/l as being suggestive of the diagnosis.

Baker (1970), reviewing the causes of feline alopecia, felt that feline hypothyroidism was uncommon and should be diagnosed on the basis of low serum PBI and hypercholesterolaemia.

Berry and Mosier (1971) reported that the aetiology of feline hypothyroidism was either thyroid atrophy or iodine deficiency. Clinical signs included bilaterally symmetrical alopecia of the lateral neck, thorax and abdomen, the remaining hair being dry and lustreless, and the skin dry and scaly. Like Conroy (1968), they also based their diagnosis on a plasma cholesterol of more than 3.9 mmol/l.

Doering (1974) felt that the serum cholesterol concentration was not useful as an indicator of hypothyroidism as it could be

affected by a number of disorders. In a subsequent report (Doering, 1976), he commented that, "A T4-thyroxin level of less than 12.9 nmol/l might be significant for consideration of hypothyroidism in cats", but no data were presented.

Austin (1975), without citing references, reported that feline hypothyroidism was not common but had been diagnosed. Clinical signs included obesity, lethargy, poor appetite, a tendency to seek warm areas and bilaterally symmetrical alopecia with or without hyperpigmentation. The serum T4 concentration was considered to be a better diagnostic test than the PBI concentration. The recommended therapy was dessicated thyroid or sodium laevothyroxine (doses not specified) but even if this was otherwise successful, the skin lesions might not completely resolve. The disease entity described by this author does not correspond to any definitively described elsewhere in the literature.

Scott (1975b) reported that hypothyroidism results from thyroid aplasia. The presenting signs were the same as those of experimental iodine deficiency but specific case details were not presented.

Lorenz and Cornelius (1976) did not describe the physical signs of feline hypothyroidism but stated that T4CPB before and after an unspecified dose of TSH by an unspecified route was the preferred method of confirming the diagnosis. Non-specific tests such as a complete blood count and serum cholesterol concentration should be used only as indicators of the hypothyroid state and not as confirmatory tests.

Martin and Capen (1983) agreed with other authors that feline hypothyroidism was uncommon. They presented photographic evidence

of what they termed, "hypothyroidism in a cat", which was accompanied by obesity and failure to groom and in which the serum T4 and T3 concentrations were "markedly reduced". No further details of this case were given but serum T4 and T3 concentrations of below 12.9 nmol/l and 0.92 nmol/l respectively were said to support a diagnosis of hypothyroidism. However, in other species, a number of non-thyroidal states may reduce the total serum thyroid hormone concentrations (see Section 3.6), and in the absence of other diagnostic tests, subnormal values fail to confirm hypothyroidism.

Sousa and Ihrke (1983), while commenting that a well-documented case of feline hypothyroidism had not been reported, stated that suspected cases were obese and lethargic with a poor haircoat. They recommended basing the diagnosis on the history, physical examination, serum T4 and T3 concentrations before and after TSH stimulation, and characteristic skin histology.

The only confirmed case of feline hypothyroidism was reported by Arnold et al. in 1984 in a 14-week-old, entire female, "European" short-haired cat presented to them because of constipation. The kitten was stunted with short legs and an enlarged head. The tongue was large and sometimes protruded from the oral cavity and the teeth were underdeveloped. The haircoat was present all over the body but consisted mainly of undercoat with primary hairs being scattered throughout. The cat did not move spontaneously, refused to play with other kittens and appeared dull. The body temperature was normal but there was a marked bradycardia (88 beats per minute). Radiographic investigation revealed almost complete absence of ossification centres of the long bones, there was a mild anaemia and the serum T4 was undetectable before and after three

daily injections of TSH. Post-mortem examination revealed thyroid lobes measuring 15 mm by 4 mm by 4 mm. Histological examination of the thyroid showed the presence of few follicles, little colloid, and tall epithelial cells containing slightly irregularly-shaped nuclei characteristic of a TSH-stimulated gland. The liver contained large amounts of glycogen. The authors attributed the condition to a congenital defect in thyroid hormone biosynthesis.

There have been two general reports on feline secondary hypothyroidism. Austin (1975) commented that if hyperpigmentation is present with other signs of hypothyroidism, pituitary dysfunction may be the initiating factor. Martin and Capen (1983) stated that secondary signs of hypothyroidism might result from pituitary tumours but could be alleviated with oral T4. None of these authors gave specific case details in these contributions.

The literature on feline hypothyroidism has also been reviewed by Scott (1980), Randolph and Jorgensen (1984) and Wilkinson (1984).

With the exception of the report by Arnold et al. (1984), the other descriptions of naturally-occurring "hypothyroidism" appear to have been based on supposition and have assumed that the clinical condition in cats must resemble that in dogs and man. Where laboratory tests have been carried out, they have not been sufficiently specific to confirm the diagnosis, leaving a case of naturally-occurring, acquired, feline hypothyroidism yet to be described.

Experimentally and therapeutically-induced cases of feline hypothyroidism

Feline hypothyroidism has been induced experimentally by

feeding diets deficient in iodine and by the administration of ^{131}I or methylthiouracil, and as a consequence of the treatment of feline hyperthyroidism.

Scott and her co-workers reported extensively on the effects of feeding raw or cooked bovine heart exclusively or with various supplements. The heart contained low quantities of iodine (10ug to 80ug per 100g dry weight) and vitamin A and had a low calcium: phosphorous ratio of approximately 0.05 (Scott, Greaves and Scott, 1961).

After feeding heart to ten to 14-week-old kittens for eight weeks, the thyroids became hyperaemic, hypertrophied and hyperplastic (Greaves, Scott and Scott, 1959). Histologically, the thyroids of kittens receiving heart alone had reduced or absent colloid, uniformly small follicles with one or more layers of columnar cells and numerous dilated interfollicular capillaries (Greaves, Scott and Scott, 1959). The condition was progressive, the severity depending on the length of time on the diet. After 30 weeks, little colloid remained and the thyroids began to show atrophic, fibrotic changes in place of hyperplasia (Scott and Scott, 1960). Supplementation of the diet with sufficient calcium, as gluconate or carbonate, to give a calcium: phosphorous ratio of 1.0 reduced the degree of thyroid hyperplasia that developed, even after eight months on the diet. Supplementation with 50 ug iodine daily (as potassium iodide) prevented gross enlargement of the thyroid and 100 ug daily completely prevented the hyperplastic changes (Scott, Greaves and Scott, 1961). When the diet was supplemented with iodine, the urinary and faecal losses of calcium were reduced, suggesting there may be a synergistic relationship between the

requirement for iodine and calcium in the cat (Roberts and Scott, 1961).

Clinical signs of hypothyroidism due to iodine deficiency were cessation of growth, sparse, short haircoat, thickened skin and broadening of the head due to oedema. Affected cats moved slowly and were affectionate and gentle (Scott, 1966, 1975a). The signs reported are very similar to those of a single case of goitrous hypothyroidism and dwarfism described by Arnold et al. (1984). Although many animals showed no sexual activity, females on the borderline of deficiency could conceive and carry foetuses to term or beyond. Subsequently, parturition was difficult and the kittens tended to have congenital defects such as open eyes and cleft palates (Scott, 1966, 1975a). No tests were carried out to confirm the hypothyroid state in these cases.

The clinical signs of hypothyroidism due to iodine deficiency were reviewed by Wilkinson (1984).

Dohan and Lukens (1938), in their investigations into feline diabetes, surgically thyroidectomised 19 cats. Wortis and Wortis (1938) experimentally thyroidectomised an unspecified number of cats by an unspecified (possibly surgical) technique. Neither group carried out tests to confirm hypothyroidism.

There are a number of reports of the induction of hypothyroidism in healthy cats using ^{131}I (Table 3.13). Crawford (1961) administered two doses of this radioisotope. He reported that attempts to collect blood prior to treatment were so difficult, even with the help of pentobarbitone anaesthesia, that they had to be abandoned. However, he succeeded in collecting blood at the time of sacrifice. Treated cats showed few signs. Their coats were ruffled

and poorly groomed and the cats spent more time sitting in their litter trays.

At post-mortem examination, three cats appeared to be totally thyroidectomised while three had residual thyroid tissue which was abnormal macroscopically, but appeared histologically to be functional in two.

The results of blood examinations were compared to untreated control cats. Unfortunately, these so called "controls" were maintained under the same conditions as the test cats for only two weeks. The total red and total white blood cell counts of the ^{131}I treated animals were lower than controls. The mean haemoglobin concentration of the experimental group was also marginally, although probably not significantly, lower than the controls. The PBI and cholesterol concentrations of the two groups were not statistically compared.

Buccino et al., (1967) induced hypothyroidism in cats using a single dose of ^{131}I . The heart rates and serum PBI concentrations of treated animals were significantly different ($P < 0.05$ and < 0.01 respectively) but the serum cholesterol concentrations were not significantly different, between the treated and the euthyroid groups.

Randall and Parsons (1970) ablated the thyroid glands of six adult male cats using ^{131}I . The hypothyroid state was not confirmed by standard laboratory techniques. The cats showed skin changes (alopecia, dry or greasy seborrhoea, hyperpigmentation of the dorsal surface of the nose), abnormal grooming behaviour, hoarseness of the voice and abdominal swelling.

Pascalov-Stoenescu and Sterescu (1971) treated 17 cats orally with 25 mg/kg methylthiouracil for ten to 14 days. They assumed that they were subsequently hypothyroid but no clinical or laboratory data were reported.

In a novel approach to the induction of feline hypothyroidism, Randall and Littschwager (1967) induced surgically, stereotaxic lesions of the brainstem of six cats. They assessed thyroid function by determining the percentage urinary excretion of an injected dose of ^{131}I . The inaccuracies of this thyroid function test have been discussed in Section 3.8.

The most frequently reported cause of feline hypothyroidism has been as a result of the treatment of feline hyperthyroidism due to bilateral (or rarely unilateral) surgical thyroidectomy (Holzworth et al. 1980; Theran and Holzworth, 1980; McMillan and Scherding, 1981; Theran, 1981; Hoenig et al., 1982; Olsen, 1982; Peterson, 1982; Black and Peterson, 1983; Hoenig, 1983), ^{131}I thyroid ablation (Peterson and Becker, 1983; Peterson et al., 1983a; Peterson, 1984; Turrel et al., 1984) or by overdosage of propylthiouracil (Peterson, 1981, 1984). Despite this large number of references, clinical details of the induced hypothyroid state are almost completely lacking. Black and Peterson (1983) mentioned the occasional development of lethargy, anorexia and weakness lasting two to three months after unilateral surgical thyroidectomy and Turrel et al. (1984) commented that one of 11 cats treated with ^{131}I subsequently became lethargic and obese. These references are reviewed in detail in Section 3.11.

Hypothyroidism and feline endocrine alopecia

The aetiology of feline endocrine alopecia is unknown (Scott,

1980). However, Martin and Capen (1975) equated the condition to hypothyroidism, Doering and Jensen (1973) and Breen and Reedy (1975) felt that it might involve thyroid hypofunction and Thornton (1963), Anon. (1966), Wilkinson (1966), Muller and Kirk (1969), Austin (1975), Muller and Kirk (1976), Thoday (1981), Muller, Kirk and Scott (1983), Sousa and Ihrke (1983), Kunkle (1984), Thoday, Seth and Elton (1984) and Thoday (1985) have reported that the condition may respond to treatment with thyroid hormones. These references are reviewed in Section 3.13.

Treatment of feline hypothyroidism

Dessicated thyroid, L-thyroxine (laevothyroxine sodium, T₄), L-triiodothyronine (liothyronine sodium, T₃) and combinations of T₄ and T₃ have all been used to treat naturally-occurring or induced cases of feline hypothyroidism. Experimental iodine deficiency has been treated with potassium iodide.

Naturally-occurring cases. Alleged cases of hypothyroidism have been reported to respond to dessicated thyroid at doses of 33 mg to 65 mg/day (Scott, 1980), 30 mg to 120 mg/day (Joshua, 1971), 60 mg to 120 mg/day (Joshua, 1965, 1979) and 2g/day (Berry and Mosier, 1971). Joshua (1971) commented that the drug was well-tolerated by cats and resulted in increased energy and improvement in coat quality. Tachycardia was an indication for withdrawing therapy.

Most authors report that once-daily therapy with T₄ is satisfactory, recommended daily doses being 10 ug to 30 ug (Muller, cited by Doering, 1974), 50 ug to 100 ug (Martin and Capen, 1983), 100 ug to 300 ug (Scott, 1980), and 22 ug/kg (Rosychuk, 1982). Doering (1974) reported that the half-life of T₄ in cats was 12 hours and therefore he recommended twice-daily therapy with a dose

of 1.1 ug to 2.2 ug/kg. No experimental data were reported. He also suggested, in the absence of cardiac insufficiency, what he termed "therapeutic diagnostics": the diagnosis of hypothyroidism by response to T4 therapy. Such approaches have no basis in science and little to commend them.

Scott (1980) reported a dose of 5 ug to 10 ug T3 to be satisfactory replacement therapy for feline hypothyroidism.

Induced cases. Scott (1965, 1966, 1975a) stated that 5 mg to 10 mg potassium iodide were well-tolerated by normal cats which excreted the excess in urine. However, cats made hypothyroid by experimental dietary iodine deficiency showed toxic side-effects of anorexia, fever, weight loss, a rapid pulse and tachypnoea when treated with the same dose. She therefore recommended treating such cases "cautiously".

Crawford (1961) treated his experimental hypothyroid cats with either 7 mg, 16 mg or 33 mg dessicated thyroid. No signs of toxicity appeared, even after prolonged periods of treatment with what the author thought were doses which were large by human standards. However, he did not state whether his treatment was successful.

Randall and Parsons (1970) used a daily intramuscular dose of 0.04 mg of a mixture of T4 and T3 in a ratio of three to two, to treat experimental hypothyroidism. They determined the "correct" dose by treating euthyroid cats with the preparation until they began to lose weight and then marginally reduced the dose so that normal weight was maintained.

T4 has been used by a number of different regimes to prevent or treat hypothyroidism arising from the treatment of feline hyperthyroidism. Peterson and Yoshioka (1983) recommended a daily

dose of 50 ug T4. Holzworth et al. (1980), Theran (1981) and Olsen (1982, 1983) used 50 ug to 100 ug T4 per day. The currently accepted standard replacement therapy is 100 ug T4 per cat once daily (McMillan and Scherding, 1981; Peterson, 1982; Birchard, Peterson and Jacobson, 1983; Peterson, 1983, 1984; Peterson, Birchard and Mehlhaff, 1984), although other authors have recommended twice-daily dosage of 100 ug (Theran and Holzworth, 1980; Hoenig et al., 1982; Black and Peterson, 1983) or 22 ug/kg (Hoenig, 1983).

Holzworth et al. (1980) have suggested that where plasma T3 concentrations remain low despite adequate T4 concentrations, 30 ug T3 three times daily or an unspecified combination dose of T4 and T3 may be used.

Despite these numerous reports, no carefully controlled studies of thyroidal kinetics in normal or hypothyroid cats have been published. Until such work is carried out, the correct thyroid hormone dosages for the treatment of feline hypothyroidism remain a subject of conjecture.

3.13 FELINE ENDOCRINE ALOPECIA

Feline endocrine alopecia (FEA) has also been termed sexual alopecia (Joshua, 1958, 1965, 1971, 1979; Baker, 1974b; Scott, 1980, 1981), hormonal alopecia (Wilkinson, 1966), hypoandrogenism (Conroy, 1968), castrate or castration alopecia (Baker, 1970; Daykin, 1971; Austin, 1975; Scott, 1975b, 1980, 1981; Keep, 1981), gonadal alopecia (Berry and Mosier, 1971; Scott, 1975b, 1980, 1981; Keep, 1981), senile alopecia (Daykin, 1971; Scott, 1975b, 1980, 1981); perineal hair loss (Chesney, 1976), and endocrine dermatitis

(Keep, 1981). FEA is the currently preferred term for the condition.

With the exception of the report on FEA by Scott (1975b), all other descriptions of the condition have appeared in review articles and have been unsupported by detailed data. This has led to conflicting claims about aetiology, predispositions and therapy of this condition.

The prevalence of the condition is disputed, with Scott (1980) regarding it as one of the more frequently seen dermatoses of cats and Sousa and Ihrke (1983) reporting it to be uncommon. Wilkinson (1981) reported geographical differences in prevalence.

Conroy (1964) reported that FEA was most common in Siamese cats. However, with the exception of one other case in a Siamese (Kirk, 1980) all other reports of FEA have been in non-pedigree animals or occasional pedigree crosses (Scott, 1975b, 1981; Thoday, 1981, 1985).

Breed, sex and age predispositions

The majority of authors report that the condition is confined to neutered individuals (Dall, 1958; Joshua, 1958, 1965; Anon., 1966; Wilkinson, 1966; Conroy, 1968; Muller and Kirk, 1969; Baker, 1970; Berry and Mosier, 1971; Daykin, 1971; Joshua, 1971; Doering, 1972, 1973; Doering and Jensen, 1973; Scott, 1975b; Muller and Kirk, 1976; Joshua, 1979; Scott, 1980, 1981). However, other workers have reported that entire cats are occasionally affected (Baker, 1974c; Austin, 1975; Thoday, 1981), and Kirk (1980), in a series of 34 cases, showed a "relatively even distribution" between entire and neutered individuals. Because the majority of mature cats seen at veterinary clinics are neutered, the

proposed predisposition of neutered animals in most reports may be apparent rather than real.

Similar confusion exists in the literature as to a sex predisposition for the disease. Anon. (1949), Joshua (1958, 1965), Wilkinson (1966), Berry and Mosier (1971), Joshua (1971), Doering and Jensen (1973), Baker (1974b and c), Austin (1975), Joshua (1979) and Thoday (1981, 1985), reported no sex predisposition. However, Scott (1975b, 1980) and Muller, Kirk and Scott (1983) reported that 90 per cent of their cases were neutered males. Other authors (Comben, 1953; Conroy, 1968; Muller and Kirk, 1969; Daykin, 1971; Doering, 1973) agreed that neutered males were more commonly affected but did not present data.

The age range of affected animals has been reported as two to ten years, with a mean of six years (Scott, 1975b); two to 12 years, with a mean of six years (Scott, 1980; Muller, Kirk and Scott, 1983), "more than two or three years" (Kirk, 1980), any age (Scott, 1981), and old cats (Thornton, 1963; Anon., 1966; Daykin, 1966; Joshua, 1979).

Aetiology

Despite the many endocrine synonyms for the disease, FEA is, in fact, a disease of unknown aetiology (Scott, 1980), but is presumed to result from hormonal changes because it usually responds well to a number of hormonal therapies (Thoday, 1981). Proposed causes include biotin deficiency (Anon., 1949), neutering at a premature age (six to 12 months) (Kral, 1959; Kral and Schwartzman, 1964; Scott, 1980, 1981), "endocrine changes" (Thornton, 1963; Keep, 1981), insufficiency of androgens in neutered males (Conroy, 1968; Doering, 1972, 1973; Austin, 1975) or oestrogens in neutered females (Conroy,

1968), unspecified sex hormone deficiencies (Joshua, 1971; Breen and Reedy, 1975; Doering, 1976; Scott, 1980; Sousa and Ihrke, 1983; Wilkinson, 1984), sex hormone imbalances (Baker, 1974c; Scott, 1980; Muller, Kirk and Scott, 1983; Sousa and Ihrke, 1983; Wilkinson, 1984), hypothyroidism (Martin and Capen, 1975), deficiencies of both sex and thyroid hormones (Doering and Jensen, 1973; Breen and Reedy, 1975) and the feeding of certain diets (Wilkinson, 1981).

Chesney (1976) proposed that FEA, miliary eczema, neurodermatitis and eosinophilic granuloma might all be forms of the same disease because they responded to megestrol acetate, and that the underlying cause was a hypersensitivity response, probably to fleas.

Recently, Ihrke (1982) and Sousa and Ihrke (1983) have also questioned the existence of FEA as a separate disease entity suggesting it was a manifestation of psychogenic alopecia and dermatitis. Kunkle (1984) agreed and felt that because the wide variety of drugs used to treat the condition all had mood-altering capabilities and some had anti-inflammatory actions, a favourable response did not necessarily prove an endocrine deficiency.

Kral (1959) and Kral and Schwartzman (1964) suggested that the reason only certain prematurely neutered cats developed FEA was deficient adrenocortical production of androgens and/or oestrogens. The interval between neutering and development of the condition is very variable (Thoday, 1981, 1985). Austin (1975) quoted a period of "a year or so" with respect to castrated males, while Scott (1975b) reported a range of one to nine years (mean five years) in a series of 20 cases.

Clinical features

Despite the considerable dispute about the aetiology of FEA, all authors are agreed on its clinical signs and the nature of the condition's progression. FEA is characterised by bilaterally symmetrical alopecia which usually begins over the perineum, the genital area, the ventral tail-base, the posterior or medial thighs or the ventral abdomen. In addition, the fore-legs (elbow to carpus) are commonly affected, sometimes early in the condition. There is diffuse thinning of hair, rather than complete alopecia. In long-standing cases, hair may be lost over the ventral two-thirds of the lateral abdomen and, less commonly, the thorax, but the dorsum is always spared. The division between affected and non-affected areas may be very well demarcated. Remaining hairs in affected areas are easily epilated and microscopically show no evidence of the free end of the shaft being broken, as may happen in self-inflicted alopecia. In most cases, the skin is otherwise macroscopically normal and there is no pruritus, but a very occasional case may show areas of erythema and mild to moderate irritation. Kunkle (1984) reported that grooming of telogen hairs may result in hairballs and gastrointestinal upsets in FEA.

Investigative procedures

Currently the diagnosis of FEA is based on the history, physical examination, elimination of other, clinically similar conditions, and response to therapy. Histopathological examination of affected skin reveals that most hair follicles are in the telogen phase and often devoid of hairs (Scott, 1980). However, such changes are non-specific as Kunkle (1984) reported that, in a study of three healthy cats over a six month period, the percentage of primary hairs in telogen was as high as 96 per cent.

Kirk (1980) reported that one male castrate had a markedly elevated serum progesterone concentration, and serum oestradiol concentrations above that of eight healthy male castrates. The serum testosterone concentration was normal for male castrates. Three affected neutered females had slightly increased serum oestradiol concentrations but normal serum progesterone and testosterone concentrations compared with five healthy spayed females. As the author commented, these results are most surprising in view of the favourable response of some cases of FEA to progestogens and testosterone. No other reports of sex hormone assays in the condition have been published.

Treatment

A large number of hormonal and some non-hormonal treatments have been recommended for the treatment of FEA.

Testosterone implants of 25 mg to 50 mg per cat have been reported to be effective in neutered males (Dall, 1958; Daykin, 1971) and both sexes (Joshua, 1965; Anon., 1966; Wilkinson, 1966; Baker, 1970; Joshua, 1971; Baker, 1974b; Keep, 1981; Thoday, 1981; Wilkinson, 1981, 1984; Thoday, 1985, 1986). Joshua (1965, 1971) found that oral administration or injection of androgens was ineffective in most cases but other authors disagreed. Oral methyltestosterone at dosages (per cat) of 1 mg to 2 mg daily (Anon., 1966), 5 mg daily (Wilkinson, 1966) and 0.5 mg to 1 mg daily (Muller and Kirk, 1976), and depot-injectable testosterone at doses of 0.5 mg/kg (Berry and Mosier, 1971) or 5 mg to 10 mg per cat (Kirk, 1980) have been recommended. Oral or depot-injectable testosterone therapy at unspecified dosages has also been used by Thornton (1963), Baker (1970), Daykin (1971), Whitehead (1971), Austin, (1975) and Breen and Reedy (1975).

In affected female cats, oral stilboestrol has been recommended at dosages (per cat) of 0.25 grains every third day for three weeks (Dall, 1958), 0.25 mg to 0.5 mg every other day (Anon., 1966), 1 mg twice weekly (Berry and Mosier, 1971), 0.1 mg daily reducing to once or twice weekly (Breen and Reedy, 1975) and unspecified dosages (Thornton, 1963; Whitehead, 1971). 0.25 mg to 0.5 mg stilboestrol may also be used to treat affected male cats (Anon., 1966). Repositol injections of 0.25 mg/kg diethylstilboestrol have been recommended by Berry and Mosier (1971). All authors agree that stilboestrol implants should be avoided as female cats subsequently show persistent oestrus (Dall, 1958; Joshua, 1965; Anon., 1966). Wilkinson (1966) warned against the use of stilboestrol by any route because of possible fatal hepato-biliary disease.

While Wilkinson (1981) reported that he had had no therapeutic success using megestrol acetate, most other authors have found progestational compounds to be particularly useful in the treatment of FEA. Megestrol acetate at initial dosages per cat of 2.5 mg daily (Sousa and Ihrke, 1983), 2.5 mg to 5 mg daily (Wilkinson, 1984) or every other day (Scott, 1980, 1981), 5 mg daily (Kirk, 1980) or every second or third day (Thoday, 1985, 1986) or unspecified dosage (Austin, 1979), has been recommended until hair growth is complete.

Other progestational compounds that have been used successfully include depot injections of progesterone at an unspecified dose (Daykin, 1971), 2 mg to 4 mg/kg (Kirk, 1980) and 2.2 mg to 22 mg/kg (Scott, 1980, 1981), and medroxyprogesterone acetate at doses of 20 mg/kg (Scott, 1979), 25 mg to 90 mg/kg (Keep, 1981), or 50 mg to 175 mg/lb (Muller, Kirk and Scott, 1983). In all instances, animals are

re-examined after six weeks and the administration repeated if required. Progesterone implants (dose unspecified) have also been recommended by Daykin (1971).

Mixtures of various sex hormones have been employed to treat FEA. Doering and Jensen (1973) and Doering (1976) recommended a depot combination of 0.1 mg to 0.5 mg oestradiol and 2.5 mg to 12.5mg testosterone to either sex, with a second administration after three weeks. Scott (1975b) reported that 18 of 20 affected cats responded to depot combination injections of 12.5 mg testosterone and 0.625 mg stilboestrol. The remaining two cats required a second administration after six weeks. Similar regimes have also been recommended by Austin (1979), Scott (1980, 1981), Kirk (1980), Keep (1981) and Muller, Kirk and Scott (1983).

Although Scott (1975b) reported that FEA cats were not hypothyroid, some authors have shown that cases respond well to thyroid hormones. Thyroid extract at daily dosages of 0.5 grain/cat and 1 grain/4.6kg was recommended by Wilkinson (1966) and Anon. (1966) respectively. L-thyroxine sodium has also been found to be effective. Muller, cited by Muller and Kirk (1976) administered 300 ug T4 daily for three months and subsequently once weekly. Keep (1981) used the same initial dosage but a maintenance dosage of 10 ug to 30 ug/day. Thornton (1963) and Austin (1979) recommended thyroid hormone replacement therapy but further details were not given.

Combinations of sex hormones and thyroid hormones have also been used to treat FEA. Muller and Kirk (1969) used a combination of either 2 grain dessicated thyroid or 100 ug to 300 ug L-thyroxine sodium daily for three to six months, together with 2 mg oral testosterone to neutered male or 0.1 mg oral stilboestrol to

neutered female cats daily. The sex hormones were discontinued temporarily if males began to spray urine or females showed signs of oestrus. Berry and Mosier (1971), Doering (1972, 1973) and Breen and Reedy (1975) reported similar treatment regimes. However, the report from Doering contradicts others from the same author (Doering and Jensen, 1973; Doering, 1976) which stated that thyroid replacement had not been attempted. Baker (1974b) successfully used a combination of 1 mg/kg thyroid extract daily for "several months" together with a 25 mg subcutaneous implant of testosterone.

Whichever hormone or combination is used, all authors agree that continuous low dosage or intermittent high dosage is required in most cases to maintain normality.

Other recommended regimes have included biotin and other vitamin or mineral supplements (Anon., 1949; Anon., 1966; Wilkinson, 1966), Whitfield's ointment (Anon., 1949), colloidal iodine oil and glucocorticoids (Wilkinson, 1966).

3.14 AUTOANTIBODIES IN FELINE NATURALLY-OCCURRING HYPERTHYROIDISM AND RADIOIODINE-INDUCED HYPOTHYROIDISM

Naturally-occurring hyperthyroidism

Human hyperthyroidism is most commonly due either to Graves' disease or to toxic nodular goitre (Toft, Campbell and Seth, 1981).

Graves' disease is characterised by diffuse hyperplasia of the thyroid gland whereas toxic nodular goitre results from one or more hyperfunctioning, adenomatous thyroid nodules (Peterson, 1984). A Graves-like condition with associated ophthalmopathy has not been recorded in cats (Peterson, 1983, 1984). The thyroid changes occurring in most cases of feline hyperthyroidism resemble human

toxic nodular goitre, for which it has been suggested as an animal model (Peterson, Becker and Hurley, 1980; Peterson and Becker, 1983; Peterson, 1984).

Graves' disease is a well-characterised autoimmune disorder in which antibodies to the TSH receptor (Manley, Bourke and Hawker, 1974; Petersen, Smith and Hall, 1976) and changes in lymphocyte subsets with a relative deficiency of suppressor cells (Thielmans, Van Haelst, de Waele, Jonckheer and Van Camp, 1981; Sridama, Pacini and DeGroot, 1982) are recognised. In common with many other autoimmune diseases, it is associated with HLA DR3 (Farid and Bear, 1981). Toxic nodular goitre, however, has no particular HLA association and the role of autoimmunity in the condition is unclear. Thyroid specific antibodies may occur (Codaccioni, Depieds, Jean, Henry and Lebreuil, 1971) and TSH receptor antibodies can be detected using sensitive cytochemical bioassays (Smyth, Neylan and O'Donovan 1983). Changes in immunoglobulin concentrations and an abnormal response to thyroid antigen in the lymphocyte migration test have been described (Kiy, Rezkallah-Iwasso, Peracoli and Mota, 1982).

Immunological studies have not been carried out in untreated cases of feline hyperthyroidism. As discussed in Section 3.11, positive serum ANA titres have been reported in small numbers of hyperthyroid cats showing serious drug reactions to PTU. In a brief contribution, Peterson, Cavanagh and Hurvitz (1983) reported ANA titres of 1:20 to 1:40 in serum samples from four of six affected cats tested by an unspecified method. Subsequently, Peterson et al. (1984) identified serum ANA by indirect immunofluorescence using HeLa cells as the substrate and fluorescein-conjugated IgG as the

developing antiserum. Titres of greater than 1:10 were reported for five of the eight affected cats tested. None of the cats was tested for ANA before drug therapy and cessation of treatment and supportive therapy resulted in negative titres within two weeks.

Radioiodine-induced hypothyroidism

While there have been previous accounts of the ablation of the feline thyroid using radioiodine (see Section 3.12) there are no reports as to whether this procedure may induce circulating thyroid antibodies.

In man, thyroid microsomal and thyroglobulin antibodies may be induced in euthyroid subjects as a result of ^{131}I therapy (Einhorn, Fagraeus and Jonsson, 1966).

4. STANDARDISATION OF RADIOIMMUNOASSAYS FOR THYROXINE AND TRIIODOTHYRONINE IN FELINE SERUM

4.1 INTRODUCTION

Standards prepared in buffer or serum of another species may result in inaccuracies in RIA procedures because of the absence of, or differences in, protein effects in standards compared with test samples. Ideally, therefore, standards for thyroid hormone assay should be prepared in T4 and T3-free serum of the same species as the test samples. This not only attempts to equalise protein concentrations in standard and sample tubes but compensates for residual protein interference not eliminated by barbiturate buffer and ANS.

Thyroid hormones may be removed from serum in a number of ways e.g. by using activated charcoal (Peterson et al., 1982; Hoenig and Ferguson, 1983; Donne and Wildgoose, 1984), agarose-covered charcoal (Stockhill, 1979) or anion exchange resin (Salter, 1979). The latter technique is currently recommended by the UK External Quality Assessment Scheme for T4 and T3.

4.2 REAGENTS

Anion exchange resin

Strongly basic anion exchange resin was obtained in the chloride form as Dowex 2X8, dry mesh 100-200, from the Sigma Chemical Company, Poole, Dorset, England.

Protein standard and protein control serum

The protein standard used in estimating serum protein concentrations was prepared from Pentex bovine albumin (ten per cent) crystalline solution, obtained from Miles Laboratories, Stoke Poges, Buckinghamshire, England. The protein control serum was Seronorm, obtained from BDH Chemicals, Poole, Dorset, England.

Thyroxine and triiodothyronine

T4 sodium salt pentahydrate (per cent purity unstated) and T3 free acid (95 to 98 per cent pure) for use as standards were obtained from the Sigma Chemical Company.

4.3 EQUIPMENT

Filter papers

Glass microfibre filter papers (coarse filter, GF/D; fine filter, GF/F) were obtained from Whatman, England.

Electrophoresis system

Serum protein electrophoresis was carried out using the Corning AC1 film/cassette electrophoresis system. Films were scanned by a Beckman R112 scanning densitometer.

Spectrophotometers

A Cecil CE 292 UV/visible range digital spectrophotometer was used for determining total serum protein concentrations. The concentrations of the T4 and T3 standard solutions were measured using a Pye Unicam SP-8-100 ultraviolet spectrophotometer.

4.4 PREPARATION OF "HORMONE-FREE" FELINE SERUM

Collection of normal serum

Blood was collected from a number of cats which were

considered to be healthy on the basis of a physical examination, and prior laboratory screening by means of a complete blood count and serum urea, AP, ALT and cholesterol estimations. Anaesthesia was induced and maintained using halothane (Fluothane, Imperial Chemical Industries), initially via an open mask and subsequently by cuffed endotracheal tube. The animals were then bled out from the carotid arteries via polythene tubing which had previously been flushed with heparin solution, 1,000 units/ml (Heparin Injection B.P., Paines and Byrne).

The blood was collected and allowed to stand at room temperature until clot retraction had occurred. Serum was harvested by centrifugation and subsequently transferred to universal containers for storage at -40°C.

Procedure for removal of endogenous thyroxine and triiodothyronine

Endogenous T4 and T3 were extracted from pooled serum using an anion exchange resin (Salter, 1979). The pooled serum was allowed to come to room temperature and a sample taken for determination of the protein content. In order to monitor the efficiency of hormone removal, ^{125}I -T4 or ^{125}I -T3 was added to give approximately 10,000 counts/minute/ml. The resultant mixture was incubated overnight to ensure binding of the radiolabelled hormones to serum proteins.

The serum was then stirred magnetically with 300mg/ml of the anion exchange resin in an incubator at 37°C for three hours. Although the original method recommends decanting the serum at this stage, it was found that the final yield could be increased by filtration through a series of glass microfibre papers on a Buchner

funnel using first a coarse filter, followed by a double layer of a fine filter. Further resin was added in the same proportions and the stirring and filtration stages repeated. A sample of the serum was then counted and the per cent removal of radiolabelled hormones determined. A sample was retained for further protein electrophoresis. Sodium azide (0.01 per cent) as a bacteriostat was added to the extracted serum which was stored in aliquots at -40°C .

Optimisation of procedures for removal of thyroxine and triiodothyronine

In the trial stages of the technique, effects of (i) time of pre-incubation of radiolabel with serum, (ii) temperature of resin extraction, and (iii) a third extraction stage on the efficiency of the process were evaluated.

(i) Effect of time of ^{125}I -T4 pre-incubation. This was compared between two samples of the same serum. One was incubated with ^{125}I -T4 at 4°C overnight and one for 15 minutes only. The resin extraction process was then run in parallel.

The percentage ^{125}I -T4 removed from serum by resin extraction after only 15 minutes' incubation with tracer was consistently but only marginally higher than that removed after overnight incubation (Table 4.1, samples A and B). Nevertheless, as a precautionary measure, it was decided to use overnight incubation as a routine measure.

Effect of temperature. Two samples of serum were resin extracted at room temperature instead of 37°C . The results showed no significant difference in the amount of T4 removed at the two temperatures (Table 4.1, samples A and C). This indicated that there was considerable latitude in the temperature required but as the

original method specified that it be carried out at a constant 37°C, this was the condition routinely used.

Effect of an additional resin extraction stage. In three studies, a third resin extraction stage was added in order to investigate whether additional, significant quantities of T4 could be removed.

The mean ^{125}I -T4 removed after two stages was 98.23 per cent. Only a mean 0.56 per cent of radiolabel was removed in the third stage (Table 4.1). It was not felt that this small increase in extraction efficiency would add to the resultant assay accuracy.

Efficiency of resin extraction

The results of six hormone extraction studies for T4 and seven for T3 are presented in Table 4.2. Experiments 1 to 4 were pilot experiments and on a small scale. Serum from experiments 5 and 6 and 5 to 8 was used for the production of T4 and T3 standards respectively. The procedure was efficient at removing added (and, therefore, presumably endogenous) T4 and T3, with mean \pm sd values of 98.84 ± 0.54 per cent and 99.45 ± 0.35 per cent respectively. The percentage ^{125}I -T3 removed was consistently greater than ^{125}I -T4. The yield of serum was approximately 70 per cent by volume.

Effect of resin extraction on serum protein concentrations

Methods. Serum protein values of samples taken before and after the resin extraction procedure were determined and the paired samples compared with each other and with normal values published from this School (Keay, 1982).

Total protein was determined by the biuret method of Henry, Sobel and Berkman (1957) using crystalline bovine albumin as standard. The final absorbances of the protein/biuret complexes were read spectrophotometrically in 1 cm plastic cuvettes against a reagent blank at 545nm. Seronorm samples were used for quality control.

Agarose electrophoresis of serum proteins was carried out according to the procedure described by the manufacturer (Corning AC1 Film/Cassette Electrophoresis System-applications Manual). 0.8ul of each serum sample was located into preformed, numbered sample wells on the film. Each film could accommodate eight serum samples. The films were electrophoresed for 35 minutes at a constant 90 volts with fresh, refrigerated 0.05mol/l barbital/EDTA buffer, pH 8.6. After electrophoresis, the films were simultaneously fixed and stained in an acetic acid/amido black solution for 15 minutes at 37°C. After destaining in acetic acid and drying completely, the films were scanned densitometrically at 600nm.

Results. Fig. 4.1 shows the mean and sd of a number of batches of sera in which protein concentrations were checked before and after the resin extraction process. The number of batches varies because while the technique was being developed, normal serum samples were divided and extracted on different occasions. In addition, it was not always possible to separate the beta-globulin component into two groups electrophoretically and such samples have not been included.

Resin extraction resulted in a slight loss in most protein fractions, but the effects were not statistically significant as assessed by Student's paired t test ($t = 1.46$, 5d.f; $P > 0.2$).

4.5 PREPARATION AND CALIBRATION OF STOCK SOLUTIONS OF THYROXINE AND TRIIODOTHYRONINE

Thyroxine standard

L-Thyroxine sodium salt pentahydrate (87mg) was weighed at room temperature and transferred quantitatively to a 100ml volumetric flask. The solvent used was propylene glycol:water (1:1 by volume) adjusted to pH 9.0 by the dropwise addition of sodium hydroxide (1.0mmol/l). The solution was agitated gently until all the sodium salt had dissolved, then made up to the mark. The concentration of this stock solution was nominally 1.0mmol/l.

The concentration of the solution in 0.1mol/l sodium hydroxide was checked spectrophotometrically from the absorbance at 325nm assuming an absorbance coefficient of $6200 \text{ l mol}^{-1}\text{cm}^{-1}$.

Triiodothyronine standard

L-Triiodothyronine free acid (65.1mg) was weighed and made up to 1.0mmol/l as above. The concentration of the solution in 0.1mol/l sodium hydroxide was checked spectrophotometrically from the absorbance at 320nm, assuming an absorbance coefficient of $4660 \text{ l mol}^{-1}\text{cm}^{-1}$.

4.6 PREPARATION OF WORKING STANDARDS

In order to conserve extracted serum, initial dilutions of stock iodothyronine solutions were made in assay buffer using a pipette checked for accuracy prior to use, to produce an intermediate stock solution of 1umol/l. Subsequent dilutions were made into extracted cat serum in two stages to produce standards of 0, 5, 10, 20, 40, 60, 80, 120, 160, and 200 nmol/l for T4, and 0, 0.125, 0.25,

0.5, 1, 1.5, 2, 3, 4, 5 and 10 nmol/l for T3. The standards were stored in aliquots in polystyrene tubes at -40°C.

Reproducibility of standard preparation

Sufficient T4 working standards were prepared to complete the studies described here but additional T3 standards had to be produced. The second set of T3 standards was prepared from the same stock standard using a different batch of "hormone-free" serum.

Standard curves with the new and old standards were virtually superimposable, the slight difference being reflected in the pool values (Table 4.3).

4.7 DISCUSSION

Correct standardisation of RIA is essential and for this reason, standards for these assays were prepared in resin extracted cat serum. The extraction method adopted has been recommended for use with human serum as it does not affect the major thyroid hormone binding proteins (C.R. Salter, personal communication), whereas charcoal extraction as used with cat serum by Peterson et al. (1982), Hoenig and Ferguson (1983) and Donne and Wildgoose (1984) tends to remove a proportion of such proteins. The charcoal extraction technique also requires the use of an ultra-centrifuge to remove charcoal fines.

When used with cat serum, the technique showed no serious effects on protein concentrations. Albumin concentrations were sometimes lowered but alpha-1 and alpha-2-globulin levels showed little change (the major human thyroid hormone binding protein, TBG migrates in the inter-alpha-1/alpha-2 zone on electrophoresis). In

fact, the alpha-1-globulin component in nine studies and the beta-1-globulin component in five studies showed mean increases of 0.25g/l and 0.24g/l respectively. The cause of these increases is unknown. It is possible that they may fall within the experimental error limits for the method. An alternative explanation is that during the extraction process, there was some fluid component loss, in which case the protein levels post-extraction in all components are artificially elevated. A third possible explanation is that some denaturing of other protein components occurred during resin extraction so that they migrated electrophoretically in different groups.

Prior to resin extraction, the total protein concentration and the concentration of albumin were marginally below the normal respective ranges as evaluated as two sds from the mean from data published from this School by Keay (1982). Such a range includes 95 per cent of the population and inevitably there will be some healthy animals which will have protein concentrations outside this range. It might have been expected that pooled serum from a number of healthy animals would come within the 95 per cent confidence limits. However, the serum was collected from arterial blood whereas Keay's samples were obtained from venous blood and this may explain the differences.

The efficiency of resin extraction of thyroid hormones is monitored by the percentage removal of radiolabelled T4 and T3 and it is assumed that endogenous T4 and T3 behave in a similar fashion. The percentage of ^{125}I -T4 removed by the technique was only marginally greater after 15 minutes' incubation with the radiolabel than if they were incubated together overnight, indicating that the

binding of T4 to serum proteins in the cat, as in man, is an extremely rapid process (Robbins and Rall, 1957).

Several modifications of the original resin extraction method were investigated. There was no significant difference in the percentage ^{125}I -T4 removed at room temperature compared with 37°C. An additional resin stage removed a mean of only 0.56 per cent more radiolabel. Because of the possibility of protein denaturation by these altered conditions, it was felt to be inadvisable and unnecessary to employ them. T3 is less avidly bound to human serum proteins than T4, and is more easily removed by resin extraction. A similar situation is likely in feline serum and it was found that in each paired study, a greater percentage of ^{125}I -T3 was removed from the serum than ^{125}I -T4. Thus, trials with a third resin extraction stage were not repeated for T3.

After four pilot experiments for T4 and three for T3, a minimum of 98.5 per cent T4 and 99.1 per cent T3 had been removed from the serum. It is generally accepted in human medicine that serum is satisfactory for standard production if greater than 95 per cent of these iodothyronines have been removed. In fact, in full-scale studies to produce serum in bulk for standard production, means of 99.45 per cent T4 and 99.70 per cent T3 were removed. This is superior to the best results obtained by the U.K. External Quality Assessment Scheme for T4 and T3 in studies on human serum (C. R. Salter, personal communication) and may reflect less avid protein binding of these hormones in cats than in man.

By counting samples of serum after the first and second resin extraction stages, it was shown that means of 96.76 per cent T4 and 98.46 per cent T3 were removed initially, leaving means of only 1.61

per cent and 0.72 per cent respectively to be removed by the second stage. By filtering rather than decanting the serum at each stage, the yields could be increased. It is obviously essential to maximise such yields when working with animals with relatively small blood volumes such as the cat.

5. DEVELOPMENT OF RADIOIMMUNOASSAYS FOR TOTAL THYROXINE AND TOTAL TRIIODOTHYRONINE IN FELINE SERUM

5.1 INTRODUCTION

A RIA is defined by three components: the radiolabelled species, the antiserum and the separation system. In these studies, ^{125}I -T₄ or ^{125}I -T₃ and sheep anti-T₄ or anti-T₃ defined the first two components. An important task, therefore, was to define the separation system.

The important requirements of a separation system are that it should:

(i) completely separate the antibody-bound and free forms of the labelled analyte (i.e. low misclassification error).

(ii) give a low "non-specific" binding (i.e. binding in the absence of the primary antiserum).

(iii) show minimum drift with exposure of incubates to separation reagents (i.e. reach equilibrium and not cause disruption of the antigen-antibody complex).

(iv) be rapid and convenient.

Many methods have been described but they fall into the broad categories of:

(i) Solvent precipitation: e.g. polyethylene glycol (PEG) which precipitates IgG.

(ii) Absorption: e.g. activated charcoal which absorbs the free analyte.

(iii) Molecular sieving: on sephadex gels, either chromatographically or in batch mode.

(iv) Immunology: using a second antibody directed against the IgG of the species in which the first antibody was raised (the double antibody technique).

(v) Solid phase methods: in which either the first or second antibody is conjugated to an insoluble support.

In these studies, both solid phase first antibody and double antibody techniques were investigated.

All RIAs require optimisation to ensure an assay response which is appropriate to the type of sample and the concentration range of interest, and a consistent response in the face of possible variations in reagent concentration. After optimisation, it is necessary to validate each assay by demonstrating satisfactory accuracy, precision and sensitivity.

This chapter describes the investigation of two RIAs, using different separation systems, for the measurement of feline total serum T4 and T3. The difficulties encountered with the solid phase system are detailed and subsequently the optimisation of the double antibody RIA methods selected for routine use are described in detail. The methods chosen and their validation are described in Chapter 6.

5.2 REAGENTS

Anti-thyroxine and anti-triiodothyronine sera

Sheep anti-T4 and sheep anti-T3 sera were obtained from the Scottish Antibody Production Unit (SAPU), Carlisle, Lanarkshire, Scotland. These antisera were pools of selected bleeds from sheep immunised with T4 and T3 respectively which had been conjugated to human serum albumin by the carbodiimide method. Cross-reactivities

on a molar basis were 2.5 per cent for T3 with the anti-T4 serum and 0.25 per cent for T4 with the anti-T3 serum. The lyophilised antisera were reconstituted with distilled water and allowed to stand at room temperature for one hour. The resulting solutions were stored in aliquots at -20°C until required.

Cellulose-linked anti-thyroxine and anti-triiodothyronine

Microcrystalline cellulose was obtained from E. Merck (Darmstadt, Germany), activated with cyanogen bromide and covalently coupled with anti-T4 or anti-T3 serum according to the method of Seth, Rutherford and McKenzie (1975). The stock preparation was stored at 4°C.

Donkey anti-sheep serum and non-immune sheep serum

Donkey anti-sheep serum (DAS), for use as a precipitating antiserum, and non-immune sheep serum (NSS) for use as a "carrier" were supplied in liquid phase by SAPU and stored in aliquots at -20°C. The DAS was a pool of selected donations from donkeys immunised with a mixture of purified normal sheep IgG.

Radiolabelled thyroxine and triiodothyronine

^{125}I -labelled T4 of specific activity greater than 34.5 MBq/nmol, and ^{125}I -labelled T3, of specific activity greater than 28.9 MBq/nmol were obtained from the Radiochemical Centre, Amersham, Buckinghamshire, England. These reagents were in ethanol/water (3:1) solution.

8-anilino-1-napthalene-sulphonic acid (ANS) ANS was obtained from the Sigma Chemical Company.

Assay diluents

Solid phase techniques. The assay diluent was 0.1mol/l

sodium glycinate buffer, pH 10.5 containing 0.2 per cent gelatine and 0.01 per cent thiomersal. The stock solution was stored at 4°C.

Double antibody techniques. The assay diluent was 0.05mol/l sodium barbiturate buffer, pH 8.6, containing 0.01 per cent sodium azide as a bacteriostat. The stock solution was stored at 4°C. In order to prevent adsorption of thyroid hormones to equipment surfaces, 0.1 per cent bovine serum albumin (Miles Laboratories) was added immediately before use.

5.3 EQUIPMENT

Assay tubes

All RIAs were performed in duplicate in 12 x 75mm polystyrene tubes (Sarstedt, Beaumont Leys, Leicester, England).

Pipettes

Sampling of serum and dispensing of reagents was carried out using a Giford Automatic Pipetter/Diluter and a Hamilton Microlab M automatic pipette.

Centrifuge

The separation of free and antibody-bound activity was performed on a Heraeus Christ 6.4 refrigerated centrifuge. A multiheaded carrier was used, giving a centrifugal force of 3,000g at 3,000 revolutions per minute (rpm).

Gamma counter

A Nuclear Enterprise NE 1600 was used. The background count (^{125}I channel) was less than 50 cpm, and regular checks were performed to ensure equivalence between channels.

Computer facilities

Data on the paper tape output from the NE1600 was entered via a Hewlett Packard paper tape reader (Model 9833A) and a Hewlett

Packard desk-top calculator (Model 9821A) for calculation of RIA results. This was done using a four parameter logit-log fit for the standard curve (courtesy of Dr. A. Smith).

5.4 INVESTIGATION OF SOLID PHASE METHODS FOR THYROXINE AND TRIIODOTHYRONINE RADIOIMMUNOASSAY

Introduction

The assay design was similar for both T4 and T3 and was based on the methods described by Seth, Toft and Irving (1976). In these techniques, the antibody is made insoluble by covalent coupling with microcrystalline cellulose. Use of antibodies in this form permits antibody-bound and unbound radiolabelled T4 and T3 to be easily separated by centrifugation. Although theoretically robust and giving high precision, the solid phase T3 assay proved to be unsuitable and only brief details of the techniques are given here.

Methods and results

Optimisation of anti-thyroxine antibody dilution. The concentrations of other reagents and reaction conditions were initially based on those used in the assay of human T4 and were as follows: ^{125}I -T4, 35,000cpm; ANS, 250ug per tube; 0 or 80nmol/l standards, 25ul; final reaction volume, 725ul.

Suspensions of stock solid coupled T4 antibody were prepared in assay diluent in doubling dilutions such that the final dilution in the incubates would range from 1:145 to 1:74,250. Titre curves were set up using 0 and 80nmol/l standards. (The anticipated normal feline serum T4 concentration was up to 80nmol/l). After incubation at 4°C for 36 hours, pre-wash solution (0.1nmol/l saline containing 0.06 per cent Brij) was added to all tubes which were then

centrifuged at 1,500g for 15 minutes. After decanting the supernatant to waste, the antibody-bound ^{125}I -T4 was counted. Graphs of per cent ^{125}I -T4 bound were plotted against antibody dilution. The optimum antibody dilution was subsequently interpolated as the largest difference in per cent ^{125}I -T4 bound between the two sera at any antibody dilution. This was found to be 1:8,700 final dilution (1:6,000 initial dilution).

Optimisation of anti-triiodothyronine antibody dilution.

This was performed as above with the following modifications:

The initial concentration of the other reagents was ^{125}I -T3, 30,000 cpm; ANS, 250ug per tube; 0 and 2.0nmol/l standards, 50ul, (the anticipated normal feline serum T3 concentration was up to 2.0nmol/l); final reaction volume, 750ul. T3 antibody final dilutions tested ranged from 1:150 to 1:77,000.

The optimum T3 antibody dilution was interpolated from graphs at 1:7,500 final dilution (1:5,000 initial dilution).

Optimisation of ANS concentrations for the solid phase thyroxine and triiodothyronine

radioimmunoassays. ANS concentrations for the T4 and T3 assays were optimised using the method described by Seth, Rutherford and McKenzie (1975). This method is described in detail for the double antibody RIA in Section 5.6. ANS concentrations tested ranged from 2,000ug per tube in doubling dilutions to 0ug per tube. The concentration of other reagents and reaction conditions were as described previously.

The optimum ANS concentrations were found to be T4, 300ug per tube; T3, 200ug per tube.

The solid phase radioimmunoassay methods for
total thyroxine and total triiodothyronine in
feline serum

The optimised reagent concentrations and the assay method are presented in Table 5.1.

Validation of solid phase radioimmunoassays
for total thyroxine and total triiodothyronine in
feline serum.

Accuracy of the solid phase RIAs was assessed by determining the recovery of known amounts of T4 or T3 added to "hormone-free" serum as described in detail in Section 6.3, to give concentrations in the range of 18.1 to 55.7nmol/l for T4 and 0.70 to 2.71nmol/l for T3. For 12 observations for T4, mean recovery \pm sd was 118 ± 16.9 per cent (range 91 per cent to 147 per cent). For 13 observations for T3, mean recovery \pm sd was 120.5 ± 25.9 per cent (range 73 per cent to 162 per cent).

Low, medium and high pools were included in each assay, prepared as described in detail in Section 6.3. Within-assay precision as judged from the high proportion of discrepant replicates was unsatisfactory.

During the validation experiments, it was found that the per cent ^{125}I -T4 and ^{125}I -T3 bound to their respective antibodies at any standard concentration varied markedly between assays. This was considered to be a possible cause of poor precision. This variability in binding was not due to leakage of antibody from the solid phase, as demonstrated by the lack of increase in binding on pre-washing antibody in buffer. It was not due to insufficient mixing of the solid phase antibody in the incubates, as standard curves obtained on shaking incubates for 16 or 24 hours at room

temperature were superimposable, but showed lower binding than the curves routinely obtained after a 36 hour incubation at 4°C.

Discussion

In solid phase RIA, the antiserum is attached to a solid support medium either by adsorption or covalent conjugation. A number of techniques have been described.

The coated tube method relies on the adsorption of the antiserum to the assay tubes (usually made of polystyrene). Separation of the bound and free antigen is simply achieved by decanting the free to waste while the bound remains attached to the tube walls and is counted directly. This simple method, while superficially having much to commend it, suffers from poorer sensitivity and precision than some other systems.

With covalent conjugation, the antibody is linked to a solid phase support medium (such as cellulose, Sephadex or Sepharose) and is added as a suspension to the assay tubes. Separation of the bound component is easily achieved by centrifugation. A modification of this technique is the magnetic antibody immunoassay in which the antibody is covalently coupled to magnetic particles. Separation of the bound from the free fractions is achieved using a magnetic separator.

Optimisation of the assays for both T4 and T3 was readily achieved but both accuracy and precision were poor, particularly with the T3 RIA. This may have resulted, in part, from fluctuations in the per cent antibody bound in the 0 standard (B_0) between assays, the cause of which was not identified. Two possible causes for this were investigated. Detachment of the antibody from the support medium was ruled out by replacing supernatant fluid by assay

buffer. In addition, studies examining the possibility that the assay reactions were not proceeding to completion, by comparing shaking and room temperature incubation with static incubation at 4°C showed, contrary to expectations, reduced binding in the former.

Precision tends to decline with decreasing concentrations of analyte. Thus, poor precision in similar assays for T3 in human serum have been observed (G. J. Beckett, personal communication). As it was clearly desirable to use the same techniques for both T4 and T3, the use of double antibody methods was investigated.

5.5 OPTIMISATION OF SECOND ANTIBODY METHODS FOR THYROXINE AND TRIIODOTHYRONINE RADIOIMMUNOASSAY

Introduction

In view of the unsatisfactory precision and sensitivity of the solid phase T3 RIA, liquid phase methods were investigated. The assay design was similar for both T4 and T3 and was based on the double antibody methods described by Ratcliffe, Challand and Ratcliffe (1974) but optimised for use with feline serum.

Optimisation requires determining:

- (i) the optimum second antibody separating conditions (that is the concentrations of DAS and NSS).
- (ii) the required amount of ANS per tube.
- (iii) the optimum first antibody dilution for the required sensitivity of the assay.

Methods

Thyroxine assay. Concentrations of DAS and NSS were optimised by the procedures of Hunter (1978). The concentration of the reagents and reaction conditions were initially based on those used in the assay of human T4 and were as follows: Anti-T4 serum,

final dilution 1:13,500; ^{125}I -T₄, 35,000 cpm; ANS, 325ug per tube; "hormone-free" feline serum, 25ul; final reaction volume, 825ul.

Initial dilutions of DAS and NSS were prepared in assay diluent so that the final dilutions would range from 1:10 to 1:330 and 1:560 to 1:22,440 respectively. Each DAS dilution was tested against each NSS dilution in the form of a matrix according to the stated assay procedure. After incubation for 36 hours at 4°C, the tubes were centrifuged, the supernatant decanted to waste, the tube tops blotted dry on absorbent paper and the antibody-bound radioactivity counted.

Triiodothyronine assay. This was performed as above, with the following modifications:

The initial concentration of other reagents was: anti-T₃ serum, final dilution 1:25,000; ^{125}I -T₃, 30,000 cpm; ANS, 90ug per tube; "hormone-free" feline serum, 50ul; final reaction volume 1,050ul.

DAS and NSS were tested in all combinations from 1:10 to 1:160 and 1:500 to 1:10,000 respectively.

Results

Thyroxine radioimmunoassay. The results of this study are presented in Fig. 5.1.

The curves show excellent plateau regions and a number of DAS and NSS concentrations could have been selected. The conditions chosen were: DAS final dilution 1:96 (initial dilution 1:73) and NSS final dilution 1:5,610 (initial dilution 1:1,360).

Triiodothyronine radioimmunoassay. The results of this study are presented in Fig. 5.2.

As in the corresponding T4 study, the curves show excellent plateau regions, the conditions chosen being DAS final dilution 1:76 (initial dilution 1:29) and NSS 1:3,000 (initial dilution 1:1,680).

Discussion

The double antibody separation method relies on the finding that on antibodies, antigenic sites (Fc fragment) and antibody sites (Fab fragment) are separate. An antibody molecule may, therefore, form a complex with its antigen and then be complexed to a second antibody. In RIA, the primary antigen-antibody complex is too dilute to be precipitated. If, however, a carrier non-immune serum is added and this is followed by an antiserum raised in a second species to the gamma-globulin of the first antibody, a much larger lattice can be established and all, including the labelled antigen bound to the first antibody, can be precipitated. Separation of the bound component is achieved by centrifugation. The method gives excellent separation (e.g. one to three per cent bound in the absence of first antibody and greater than 95 per cent bound in the presence of excess antibody (Hunter, 1978)). A single experiment is sufficient to optimise a system, although this must be repeated each time a new batch of second antibody is used.

Because of the good "plateau" regions in the plotted results, various combinations of DAS and NSS concentrations could have been selected for routine assay use. DAS is relatively expensive and generally cannot be used in high dilution. Thus, without compromising maximum efficiency and reproducibility, it is usual to select the greatest dilutions of DAS and NSS compatible with maximum binding. Selection of DAS and NSS concentrations giving a "plateau"

in binding ensures that minor deviations from the concentrations chosen, due to, for example, pipetting errors or errors in reagent dilution, do not adversely affect the assay. The lower binding of labelled hormone at dilutions higher and lower than the optimum reflect conditions of antibody excess and deficiency.

During optimisation of these RIAs, it was helpful that similar assays were in routine diagnostic use with human sera locally. This provided initial information on criteria such as reagent volumes, first antibody and ANS concentrations, reaction times and temperature, and centrifugation times. These were subsequently optimised specifically for feline serum.

5.6 OPTIMISATION OF ANS CONCENTRATIONS USING "HORMONE-FREE" SERUM

Introduction

ANS is usually included in the RIA incubate in the measurement of thyroid hormone concentrations in human serum. It binds to the hydrophobic sites of serum proteins displacing T4 and T3 and makes them available for reaction with their respective antibodies, and minimises serum protein binding of radioactive T4 and T3. The mass per tube must be carefully optimised.

The object of these experiments was to determine the optimum concentration of ANS for the determination of T4 and T3 in feline serum.

Method

Thyroxine radioimmunoassay. Doubling dilutions of ANS were prepared in assay diluent so that the final mass per assay tube ranged from 1,500ug to 0ug. The final dilution of anti-T4 was 1:13,500 with those of DAS and NSS being as determined previously.

At each concentration of ANS, triplicate incubates were prepared with the addition of either "hormone-free" feline serum (25ul) or diluent (25ul). After incubation, centrifugation and counting, graphs were plotted of per cent ^{125}I -T₄ bound against mass of ANS per incubate.

Triiodothyronine radioimmunoassay. The same technique was used as above, varying the amount of ANS from 800ug per assay tube in doubling dilutions to 0ug per tube, with the final dilution of anti-T₃ serum being 1:25,000 and DAS and NSS concentrations as determined previously.

Results

Thyroxine radioimmunoassay. The results of this study are presented in Fig. 5.3.

As the concentration of ANS per tube increases, the per cent ^{125}I -T₄ bound to the T₄ antibody increases as the binding to serum proteins is inhibited. The concentration of ANS required to block serum protein binding maximally is shown by the greatest possible binding in the presence of extracted serum. If the concentration of ANS is further increased, antibody binding of T₄ is progressively inhibited. To allow a safety margin, the optimum ANS used per tube is slightly greater than that required to block serum protein binding maximally, that is 290ug per tube (Fig. 5.3). The difference in per cent ^{125}I -T₄ bound in the tubes containing diluent or extracted serum represents the residual effect of serum proteins that is not inhibited by ANS.

Triiodothyronine radioimmunoassay. The results of this study are presented in Fig. 5.4.

There is no increase in the per cent ^{125}I -T3 bound to the T3 antibody as the mass of ANS per tube increases, suggesting that ANS has no effect in this assay. When the mass of ANS is increased above 50ug per tube, blocking of radiolabelled T3 to T3 antibody begins to occur as shown by the parallel fall in the per cent ^{125}I -T3 bound in both diluent and extracted serum tubes.

Discussion

ANS has the property of binding to hydrophobic sites on protein molecules, and in doing so, appears to displace T4 and T3. The inclusion of ANS in the incubates, therefore, reduces competition from the serum proteins for binding of thyroid hormones, and is essential if accurate results are to be obtained. The weight per tube must be carefully optimised. If there is insufficient ANS, its effects as described are reduced. If present in excess, the antibody binding itself may be lowered.

The mass of ANS required in the T4 assay was 290ug per tube compared with 325ug in a comparable assay for human serum. It is perhaps surprising that there is so little difference in the requirements of ANS between assays for the two species when human serum is known to contain TBG, and a comparable high-affinity thyroid hormone binding protein in feline serum could not be demonstrated (Tanabe, Ischii and Tamaki, 1969; Bigler, 1976a).

The conclusion that ANS did not appear to be necessary in the T3 assay was surprising but may reflect these reports, although other authors have described the presence of a feline TBG (Farer et al., 1962) but of low binding capacity (Scherzinger, Guzy and Lorcher, 1972). It is possible that barbiturate, also known to inhibit protein binding, might have provided sufficient inhibition in this assay.

In order to confirm whether or not ANS could be omitted from the T3 assay, an additional experiment using normal feline sera (as opposed to the "hormone-free" serum used here) was performed.

5.7 OPTIMISATION OF ANS CONCENTRATION IN THE TRIIODOTHYRONINE RADIOIMMUNOASSAY USING NORMAL SERA

Introduction

In the absence of ANS in the RIA of T3 in human serum, the protein binding sites for T3 are not blocked and bind more of the ^{125}I -T3 than when ANS is present. This results in reduced antibody binding of radiolabel and a higher value for the T3 concentration. This study investigates whether such an effect occurs in feline serum and compares the results to those seen in samples of human serum of known thyroid hormone concentration.

Method

T3 assays were set up with and without ANS (90ug per tube). Each assay included a standard curve, the same samples of normal feline sera of unknown concentration, and human sera of known concentration from hypothyroid, hyperthyroid and euthyroid patients.

Assay conditions were: anti-T3 serum, 1:13,500 final dilution; ^{125}I -T3, approximately 30,000 cpm per tube; DAS, 1:76 final dilution; NSS 1:3,150 final dilution; final incubation volume 1,050ul. Incubation was carried out at 4°C for 36 hours, and the antibody-bound fraction separated by centrifugation and counted as before.

Results

These are presented in Table 5.2.

With human sera, the results were as anticipated with higher T3 values being recorded in the absence of ANS. These differences were highly significant as assessed by Student's paired t test ($t = 8.65$, 8d.f; $P < 0.001$). These effects appeared more obvious at low T3 concentrations. However, with cat serum, the reverse effect was found, with T3 concentrations being consistently lower in the absence of ANS than when it was included. These differences were also highly significant as assessed by Student's paired t test ($t = 7.78$, 9 d.f; $P < 0.001$). This effect was unexpected. It may have been due to the ANS depressing binding in the standard tubes (containing extracted serum) more than in the sample tubes.

The values for T3 concentrations of human sera read against both human and feline standards were also compared using Student's paired t test. The differences were highly significant ($t = 9.00$, 9 d.f; $P < 0.001$).

As ANS did not adversely affect the T3 assay, it was decided, as a safety measure, to include it in the incubate. The mass required per tube was evaluated from Fig. 5.4 as that which reduced the antibody-binding to just below the maximum obtainable in the study (90ug per tube).

Discussion

The inclusion of the protein-binding inhibitor ANS in the incubates was found to be necessary despite reports in the literature that high affinity binding proteins for thyroid hormones could not be demonstrated in cat serum (Tanabe, Ishii and Tamaki, 1969; Bigler, 1976a). As barbiturate contributes to the blocking of the binding of thyroid hormones to their carrier proteins, it was

thought that it might be possible to omit ANS from the incubate, particularly in the T3 assay. Indeed, optimisation in the presence of "hormone-free" serum suggested that ANS had no effect in the assay. However, in the absence of ANS, T3 concentrations were consistently a mean of 16.4 per cent lower and ANS was therefore included in both assays.

The explanation for the apparent discrepancy between the effects of T3 in the optimisation experiment using "hormone-free" serum and normal feline serum may lie in the binding properties of the extracted and sample sera. Clearly, the extracted serum can only approximate the composition of normal sample sera and is not identical with it. In view of the small but significant difference in results with feline sera, it was considered prudent to include ANS in the incubates for the T3 RIA. The observation that omission of ANS had a much greater effect in the assay of human, than in the assay of feline, samples provided confirmatory evidence of the relatively minor effect of ANS in the assay of T3 in feline serum.

There was a highly significant difference in T3 concentrations of human sera when determined in assays standardised and optimised for the cat and man respectively. Thus, the use of commercial human RIA kits or testing services offered by human reference laboratories should be discouraged unless the assays have been validated adequately for cats.

5.8 OPTIMISATION OF FIRST ANTIBODY DILUTIONS

Introduction

Optimisation of first antibody dilutions is necessary in order that the maximum change in signal (per cent radiolabel bound) occurs over the ranges of clinical interest.

Method

Thyroxine radioimmunoassay. Solutions of T4 antibody were prepared in assay diluent such that the final dilution in the incubates would be 1:10,300, 1:13,600, 1:20,600 and 1:28,000. Standard curves were then set up using these dilutions of first antibody and concentrations of reagents as previously determined.

Triiodothyronine radioimmunoassay. The T3 antibody dilution was optimised in the same way as for the T4 RIA using final dilutions of anti-T3 of 1:15,000, 1:20,000 and 1:25,000 and other conditions as previously determined.

All incubations were carried out for 36 hours at 4°C with separation by centrifugation.

Results

Thyroxine radioimmunoassay. The results of this study are presented in Fig. 5.5.

The best compromise for the first antibody dilution covering the anticipated hypothyroid, euthyroid and hyperthyroid ranges was a final dilution of 1:20,000 (initial dilution, 1:4,700).

Triiodothyronine radioimmunoassay. The results of this study are presented in Fig. 5.6.

The optimum first antibody concentration was evaluated as for T4 at 1:15,000 final dilution (1:8,400 initial dilution).

Discussion

The specificity of any RIA rests on the ability of the first antibody to recognise the antigen under test, and that antigen alone (any deviations from this being described as per cent cross reactivity). The interaction between antibody and antigen involves numerous weak bonds present only between closely fitting parts of

the molecules. It is the so-called N terminal of the immunoglobulin which is responsible for the binding of antigen.

Antibodies are almost bound to be heterogeneous because the antigenic response brings responses from immunoglobulins of all types. There may also be variability within the same antibodies raised against the same determinant, brought about by differences in degree of fit and area of the antigen molecule with which they combine. An antiserum with antibodies which combine closely with only a few determinants of the antigen is likely to be more specific but less tightly bound than one with a greater variety of antibodies combining with more determinants and with a wider range of fit.

The optimum first antibody dilution is that which gives the maximum change in signal over the ranges of clinical interest. These assays were directed at a clinical situation for the recognition of possible hypo and hyperthyroidism. Thus it was necessary to select the best compromise in first antibody dilutions over a wide range of T4 and T3 concentrations.

6. THE RADIOIMMUNOASSAY METHODS FOR TOTAL THYROXINE AND TOTAL TRIIODOTHYRONINE IN FELINE SERUM

6.1 TOTAL THYROXINE

Working reagents and standards

The following reagents were freshly prepared in assay diluent (0.05mol/l barbiturate containing 0.01 per cent azide and 0.1 per cent bovine serum albumin, the latter added immediately before use) for each assay.

Reagent 1. ^{125}I -T₄ (157u1) was diluted to 231ml in assay diluent and donkey anti-sheep serum (DAS, 3.2ml) added to give a working dilution of 1:73.

Reagent 2. 8-anilino-1-napthalene sulphonic acid (ANS) (110mg) was diluted to 80ml in assay diluent and sheep anti-T₄ (17u1) and non-immune sheep serum (NSS, 59u1) added to give working dilutions of 1:4710 and 1:1,360 respectively.

These volumes were sufficient for 360 assay tubes.

Thyroxine standards. T₄ working standards in "hormone-free" feline serum were prepared and stored as described in Chapter 4.

The reagents, samples and standards were allowed to come to room temperature and each was mixed well before use.

Method

- (1) All standards, samples and controls are assayed in duplicate.
- (2) The assay is arranged with tubes 1-20 for standards, with pools of low, medium and high concentrations at the beginning and end of the block of samples. A

maximum of 80 samples and pools is assayed against each standard curve.

- (3) Using an automatic diluter, pick up standard (25ul) or sample (25ul) and dispense together with reagent 1 (600ul). Dispense reagent 2 (200ul). Vortex mix.
- (4) Incubate for 36 hours at 4°C.
- (5) Centrifuge at 3,000 rpm for 30 minutes at 4°C.
- (6) Decant supernatant to waste. Blot tube tops dry on absorbent paper.
- (7) Determine antibody-bound ^{125}I in tubes by counting in a gamma counter.
- (8) Enter count results on paper tape via Hewlett Packard tape reader (Model 9883A) and calculator (Model 9821A) into computer (Hewlett Packard 9871A) for calculation of results using four parameter logit-log model for the standard curve.

A summary of the composition of the assay incubate is given in Table 6.1.

6.2 TOTAL TRIIODOTHYRONINE

Working reagents and standards

The following reagents were freshly prepared in assay diluent (0.05mol/l barbiturate buffer containing 0.01 per cent azide and 0.1 per cent bovine serum albumin, the latter added immediately before use) for each assay.

Reagent 1. ^{125}I -T3 (300ul) was diluted to 135ml in assay diluent and DAS (4.8ml) added to give a working dilution of 1:29.

Reagent 2. ANS (34mg) was diluted to 210ml in assay diluent and sheep anti-T3 (25ul) and NSS (125ul) added to give working dilutions of 1:8,400 and 1:1,680 respectively.

These volumes were sufficient for 300 assay tubes.

Triiodothyronine standards. T3 working standards in "hormone-free" feline serum were prepared and stored as described in Chapter 4.

The reagents, samples and standards were allowed to come to room temperature and each was mixed well before use.

Method

- (1) All standards, samples and controls are assayed in duplicate.
- (2) The assay is arranged with tubes 1-22 for standards, with pools of low, medium and high concentrations at the beginning and end of the block of samples. A maximum of 80 samples and pools is assayed against each standard curve.
- (3) Using an automatic diluter, pick up standard (50ul) or sample (50ul) and dispense together with reagent 1 (400ul). Dispense reagent 2 (560ul). Vortex mix.
- (4) Incubate for 36 hours at 4°C.
- (5) Centrifuge at 3,000 rpm for 30 minutes at 4°C.
- (6) Decant supernatant to waste. Blot tube tops dry on absorbent paper.
- (7) Determine antibody-bound ^{125}I in tubes by counting in a gamma counter.
- (8) Enter count results on paper tape via Hewlett Packard tape reader (Model 9883A) and calculator (Model 9821A)

into computer (Hewlett Packard 9871A) for calculation of results using four parameter logit-log model for the standard curve.

A summary of the composition of the assay incubate is given in Table 6.1.

6.3 VALIDATION OF ASSAYS FOR TOTAL THYROXINE AND TOTAL TRIIODOTHYRONINE IN FELINE SERUM

Introduction

Once a RIA system has been optimised it must be validated to determine its reliability. The criteria of assay validity are specificity, accuracy, precision and sensitivity.

Specificity is the extent of freedom from interference by substances other than the one intended to be measured (Midgley, Niswender and Rebar, 1969). Accuracy is the extent to which the measurement of an antigen in a sample agrees with the "correct" amount that is present (Midgley, Niswender and Rebar, 1969). Precision is a measure of the scatter of the individual estimates around their mean value. Sensitivity (detection limit) is the smallest amount of antigen that can be distinguished from no antigen.

Specificity

Method. Specificity may be evaluated by the extent of cross-reactivity of antibodies to chemically similar substances.

Results. For these assays, cross-reactivities on a molar basis were provided by the manufacturers of the antibodies and were 2.5 per cent for T3 with the anti-T4 serum, and 0.25 per cent for T4 with the anti-T3 serum.

Accuracy

Method. Accuracy was assessed by determining the recovery of known amounts of T4 or T3 added to "hormone-free" or normal feline sera to give concentrations in the range of 18.1 to 69.0nmol/l for T4 and 1.0 to 2.0nmol/l for T3. The T4 and T3 was added as a 2.0ul volume to a 1.0ml volume of serum, the T4 (940nmol/l) and T3 (36.6nmol/l) being prepared in diluent.

The per cent thyroid hormone recovery was calculated from the equation:

$$\text{Per cent hormone recovery} = \frac{(\text{Concentration of hormone found} - \text{Endogenous hormone concentration}) \times 100}{\text{Concentration of hormone added}}$$

Results. The results of the recovery experiments for T4 and T3 are presented in Tables 6.2 and 6.3 respectively. In both assays, recovery was excellent, mean \pm sd (number of observations) being 106 ± 5.9 per cent (n=11) for T4 and 104.4 ± 8.5 per cent (n=9) for T3.

Precision and drift

Method. Low, medium and high pools were included in each assay. The medium pool was prepared from normal feline serum and the low and high pools from this by dilution with "hormone-free" cat serum, and by the addition of known amounts of T4 and T3 in assay buffer respectively. At least one pool was included at least twice in each assay so that evidence of drift and within-assay precision could be evaluated. Between-assay precision was also studied.

Results. Data on T4 and T3 pools from which results on precision and drift were derived are given in Tables 6.4 and 6.5 respectively.

Within-assay coefficients of variation (cv) for low, medium and high pools respectively were five per cent, four per cent and two per cent for T4, and 12 per cent, five per cent and five per cent for T3.

There was no evidence of drift in either assay as assessed by Student's paired t test on pools placed at the beginning and end of the assays (T4:t=0.03, 16d.f; T3:t=0.50, 25d.f.).

Data on between-assay precision for low, medium and high T4 and T3 pools are presented in Table 6.6. The mean cvs for the T4 and T3 assays were 6.6 per cent and 12.1 per cent respectively.

Sensitivity (detection limit)

Method. Assay detection limits can be estimated as the concentration of hormone required to give a statistically significant signal in the assay. The detection limit may be calculated as the concentration of analyte which corresponds to twice the sd of the zero standard binding.

Results. Detection limits so calculated were approximately 1.3nmol/l for T4 and 0.1nmol/l for T3. In practice, a more conservative and simple approach was adopted, by taking the lowest standard (5 nmol/l and 0.13nmol/l respectively for T4 and T3) as the detection limits.

Discussion

Data on specificity, accuracy, precision and sensitivity are necessary if RIA is to be considered reliable.

Specificity may be evaluated quantitatively by the comparison of inhibition curves produced by standards and serial dilutions of normal sera, and by the extent of cross-reactivity of antibodies to chemically similar substances, and qualitatively by reproducing and measuring known biological responses in vivo (Reimers et al.,

1981). In these assays, cross-reactivities for the anti-T4 and anti-T3 sera were provided by the manufacturers (see Chapter 5).

Accuracy is ideally assessed by comparison of assay results with measurements made by a definitive "reference" method. Gas-chromatography - mass spectrometry is used to assess accuracy of some RIAs for haptens, although this technique is not yet available for iodothyronines.

A simpler, if less complete, approach is to measure recovery of added analyte, although it is important to appreciate that quantitative recovery does not ensure accuracy. Recovery experiments will reveal errors in standardisation, but not background interference if this contributes both to the spiked and unspiked samples.

By this technique, accuracy was found to be excellent with means of 106.0 per cent for the T4 and 104.4 per cent for the T3 assays.

Quality control samples of low, medium and high concentrations should be included in all RIAs to allow the calculation of within and between-assay precision. Within-assay and between-assay precision are determined by calculating the cv of a number of measurements of the same sample within one or in a number of assays respectively. Reimers et al. (1981), Hoenig and Ferguson (1983), Donne and Wildgoose (1984) and Kemppainen, Mansfield and Sartin (1984) are the only workers to have reported such data for feline T4 and T3 RIA. Hunter (1978), referring to human RIAs in general, stated that, "the precision of determination of antigen in physiological fluids is generally of the order of \pm five to ten per cent for within-assay duplication and \pm eight to 15 per cent

for between-assay replication as determined from quality control samples". In the present study, within and between-assay precision for T4 and T3 was excellent and fell easily into Hunter's criteria. The single exception was between-assay precision for the low T3 pool but this compared well with that of similar assays and reflected the low concentrations being measured.

7. CLINICAL STUDIES OF THYROID HORMONE CONCENTRATIONS IN HEALTHY CATS

7.1 INTRODUCTION

While there are a small number of reports of T4 and T3 concentrations by RIA in healthy cats, no detailed investigations have been carried out into the effects of such variables as age, sex, breed, heredity and environment. The aim of this study was to establish the normal range of feline T4 and T3 and to investigate the influence of such variables on these ranges.

7.2 METHODS

Clinical material

Blood was obtained from 319 cats (122 males, 141 females, 35 neutered males and 21 neutered females) aged between four months and 13 years. All were classified as healthy on the basis of a detailed history and physical examination. Pregnancy checks were carried out on all entire females by ballottement and those considered to be positive were excluded from the study. The majority of the animals were either domestic short or long-haired, but 23 pedigree animals of several breeds were also represented.

Sample collection and preservation

Samples were collected from conscious animals by venepuncture, usually from the jugular vein but also from the cephalic vein. After clot retraction, blood was centrifuged and the serum transferred to a clean tube for storage at -40°C. Where the volume of sample allowed, T4 and T3 were assayed on each specimen giving 298 paired

observations. Twenty samples were assayed for T4 alone and one for T3 alone.

RIAs were performed as detailed in Sections 6.1 and 6.2.

7.3 RESULTS

Distribution of values in the healthy population (all ages and both sexes)

T4 and T3 values were found to fit a normal distribution (chi-squared goodness-of-fit tests on grouped data were not significant). Figs. 7.1 and 7.2 are histograms of the total T4 and T3 concentrations respectively in the healthy population and show how the normal distribution closely describes the observed values. Table 7.1 summarises the data in terms of mean, sd, range and 95 per cent range (2.5 per cent and 97.5 per cent sample percentiles).

Three of the T4 and ten of the T3 estimations gave values that were less than the working detection limits of the assays. In each case, however, the paired T3 and T4 concentration was within the 95 per cent reference range. Data on these animals are presented in Table 7.2.

Relationship between total thyroxine and triiodothyronine concentrations (all ages and both sexes)

Individual T4 and T3 values were highly significantly correlated ($r = 0.46$, $P < 0.001$). This relationship is shown in Fig. 7.3.

Effects of age, sex and breed on hormone concentrations

Multiple regression was used to investigate the relationship of T4 and T3 concentrations to age, sex and breed. This method tests whether or not mean hormone concentrations are significantly

affected by each factor when the effect of all the others is taken into account. A quadratic term for age was included to test whether any relationship with age was linear. In view of the small numbers of pedigree cats, the analysis compared all pedigree to all domestic breeds. The former comprised ten Siamese, seven Burmese, three British blues, two Devon rex and one Persian, while there were 254 domestic short-haired and 24 domestic long-haired cats.

For descriptive purposes, animals were divided into five age groups to allow, as far as was possible, for different life stages:

- Group 1: 4 - 6 months (pre-puberty)
- Group 2: >6 - 12 months (pubertal growing animals)
- Group 3: >12 - 60 months (young adulthood)
- Group 4: >60 - 108 months (maturity)
- Group 5: >108 months (elderly)

However, multiple regression testing analyses the data according to specific age and not on arbitrary groupings.

Data on effects of sex, age and breed on total T4 and T3 concentrations are presented in Tables 7.3 and 7.4. The results of the analyses as applied separately to T4 and T3 are summarised in Table 7.5. In the case of T4, both the linear and quadratic effects of age were highly significant and there was also a significant difference in T4 concentrations between the original sexes. Fig. 7.4 illustrates mean values (\pm SEM) for T4 for each sex in the five age groups, and shows that T4 concentrations in both sexes tended to decrease with age up to approximately five years and then to rise again. Female cats of a given age tended to have higher T4 values than male cats. The effects of neutering and breed were not significant for a given age and original sex.

For T3, a similar, though less pronounced age effect was found, with the initial decrease with age being followed by a levelling out rather than an appreciable rise. The effects of original sex and neutering on T3 were not significant, but pedigree cats tended to have higher concentrations of the hormone at any given age than domestic cats (Tables 7.4 and 7.5). Fig. 7.5 illustrates the differences in mean serum T3 concentrations of pedigree and domestic cats according to age. In neither case did Siamese cats considered separately from other pedigree cats differ significantly from domestic long-haired cats for given levels of the other significant factors.

Fitted multiple regression of significant factors for T4 and T3 was:

$$T4 = 27.1 + 2.94 \text{ (if originally female)} - 0.224 \times \text{age} + 0.0019 \times \text{age}^2$$

$$T3 = 0.77 + 0.09 \text{ (if not domestic breeds)} - 0.068 \times \text{age} + 0.00004 \times \text{age}^2$$

Influence of heredity and environment on hormone concentrations

If environmental effects influence T4 and T3 concentrations, it would be expected that cats reared in the same environment would show less variation in hormone concentrations than those reared in different environments. Similarly, a genetic component to hormone concentrations would lead to related cats varying less than unrelated ones. To examine these possibilities, hormone concentrations were measured in 12 unrelated cats grouped in five environments (Table 7.6) and in 18 related cats grouped in nine environments (Table 7.7). Nested random-effect analysis of

covariance was used to test for significance between environment and between-family effects, while correcting for the previously established effect of age on T4 and T3 concentrations.

The results of these analyses are shown in Table 7.8 together with their interpretation. The significant excess variation between environmental groups for the related cats suggests that hereditary and/or environmental influences exist on both T4 and T3, but it is not clear which component predominates for T4. For T3, there appears to be a definite hereditary component.

Apparent euthyroidism with elevated thyroid hormone concentrations

Data on these cases are presented in Table 7.9.

One healthy cat (FT/NC/9/82) was found to have grossly elevated T4 and T3 concentrations. The sample was taken prior to routine dentistry and before premedicants were given. Another cat (FT/NC/3/82) had normal T4 but grossly elevated T3 concentrations. This cat had been involved in a road traffic accident three weeks before, resulting in separation of the mandibular symphysis. This was repaired immediately with wire around the lower canines, no drugs had been administered post-operatively and, according to the owner, the cat had been clinically normal for two weeks. The sample was taken prior to removing the wire, before premedication.

Because these values were grossly elevated, they were excluded from calculations of normal ranges.

7.4 DISCUSSION

Many of the reported early studies of feline thyroid function used methods which have been superseded in studies on humans

because of poor specificity, sensitivity or both. RIA is now firmly established for the investigation of thyroid disease in man. Similar techniques are applicable to the cat provided the methods are modified to allow for the much lower concentrations of circulating T4 and T3 in this species, and the different sample matrix presented by cat serum.

When this study was initiated, Reap, Cass and Hightower (1978) and Anderson and Brown (1979) were the only workers to have reported details of the RIA methods they used to determine plasma total T4 and T3 concentrations in normal cats. Following the recognition in 1979 of feline hyperthyroidism by Cotter and by Peterson, Johnson and Andrews, and its further definition as an important entity in older cats (Holzworth et al., 1980), there has been considerable interest in the biochemical investigation of this disease. There have been several subsequent reports on total T4 and T3 concentrations in normal cats but most have been simple statements of reference ranges for comparison with hyperthyroid individuals (see Table 3.4).

While others have modified RIAs for use in cats, this work describes the first RIA specifically designed for measuring thyroid hormones in cat serum. Although Donne and Wildgoose (1984) reported no significant differences for feline T4 and T3 concentrations between heparinised plasma and serum, and no effects of freezing and thawing and steroid anaesthesia, in this study, blood samples were taken from conscious animals without prior use of sedation. Only serum was used and this was separated from cells as soon as clot retraction had occurred and stored at -40°C. Standards and pools were also stored aliquoted at -40°C to avoid repeated thawing and freezing.

This study, based on 318 T4 estimations and 299 T3 estimations, is more comprehensive than any previously described (Table 3.4). The values reported here can be considered reliable as they were obtained with well-validated methods on a large population. The reference ranges from different laboratories usually vary as a result of differences in methodology and it can be confusing and hazardous to compare them. Although the mean T4 and T3 concentrations found here are in good general agreement with previously reported values, it is evident that some euthyroid concentrations in other series would be in the hyperthyroid ranges on the basis of the present data. These high values have largely been described in the earlier studies. As assay reagents become more specific for the analyte and procedures more refined, there is a tendency for reference ranges to become lower.

0.94 per cent of the determined T4 values and 3.35 per cent of the T3 values were less than the working detection limits of the assays. In each case, however, the paired T3 or T4 concentration was within the 95 per cent confidence limits for the respective assay. The assays were designed so that both thyroid hypo and hyperfunction as well as the normal ranges could be evaluated and inevitably there will be some animals which will have hormone concentrations outside the 95 per cent normal range. To have re-optimised the assays to measure the lowest concentrations of T4 and T3 found would have meant a loss in working range, at the higher concentrations. In addition, the value of the 2.5 per cent sample percentile was greater than the lowest standard, for each assay.

In this study, the serum total T3 concentration was found to be highly significantly related to the serum total T4 concentration.

While the methods of T3 production in the cat have not been established, such a relationship would be expected if its derivation were similar to that in man, where only a small proportion of circulating T3 is secreted by the thyroid, approximately 80 per cent of it being produced from extra-thyroidal mono-de-iodination of T4 (Braverman and Vagenakis, 1979).

Effect of age

Variations in thyroid hormone concentrations with age have not been reported previously in cats. In this study, total T4 concentrations were found to vary highly significantly with age in a non-linear manner, mean concentrations decreasing in both males and females until greater than 12 to 60 months and increasing thereafter. T3 concentrations also showed this non-linear decrease with age but tended to level out rather than increase at very high ages. It would be expected that T3 changes would parallel those of T4 in view of the findings that T4 and T3 concentrations were highly correlated. In man, thyroid hormone concentrations decrease with increasing age in hospital patients but not in the healthy elderly; that is, the decrease appears to be a consequence of illness rather than age per se (Olsen, Laurberg and Weiike, 1978). Cats for inclusion in this study were taken from a healthy rather than a hospital population but, nevertheless, the rise of total serum T4 concentrations in older animals was not expected.

Effect of sex

Literature reports of the variation of thyroid hormone concentrations in cats according to sex are contradictory. Most studies failed to find any difference but Bigler (1976b) reported higher concentrations in females than in males, with Anderson and

Brown (1979) finding higher concentrations in entire compared to ovariectomised animals. In man, thyroid hormone concentrations have been reported to be slightly higher in females than in males, but there is no universal agreement on this. Indeed, the tendency to higher concentrations in females may have been due to the inadvertent inclusion of some pregnant women in the series (Robbins and Rall, 1979).

In this study, an attempt was made to exclude pregnant animals by physical examination. For any given age, female and neutered female cats taken as one group tended to have significantly higher T4 concentrations than males and neutered males. No such effect was identified for T3. When age was accounted for, the effects of castration or ovariectomy were not significant for either T4 or T3.

Effect of breed

In the only report on the relationship between breed and thyroid hormone concentrations, Ling, Lowenstine and Kaneko (1974) found a significantly higher concentration of total T4 in Siamese than in domestic long-haired cats. Because the present study only included 23 pure-bred cats, data were pooled and compared with those for domestic short and long-haired animals taken together. No significant differences in T4 concentrations were found but the total T3 concentrations were significantly higher for any given age in the pedigree breeds. When the Siamese breed was compared separately with domestic long-haired cats, T4 and T3 concentrations did not differ significantly.

Effects of heredity and environment

Of particular interest was the similarity between T4 concentrations of animals kept in the same environment. T3

concentrations also showed a close similarity within a single environment, and these similarities were demonstrated statistically. However, when the animals were separated into related and non-related groups, it was not possible to show which component predominated for T4. For T3 there appeared to be a definite genetic component. Such findings have not been reported previously but they require further studies on larger numbers of animals.

Euthyroid animals with raised thyroxine or triiodothyronine concentrations

Euthyroid hyperthyroxinaemia is a well-documented syndrome in man (reviewed by Borst, Eil and Burman, 1983). The commonest cause is acquired TBG excess as a result of pregnancy (due to oestrogen-induced TBG production by the liver), non-thyroidal illness (hepatic disease with TBG leakage into the circulation, porphyria, oestrogen-producing tumours, hydatidiform mole and lymphosarcoma), and drug induction (oestrogen, narcotics, 5-fluorouracil and clofibrate).

Inherited thyroxine-binding abnormalities (increased TBG, excess albumin binding and increased TBPA) have also been described in man. In increased TBG concentrations, both total T4 and T3 are elevated, whereas in excess albumin binding and increased TBPA, total T3 concentrations are normal.

Other causes of euthyroid hyperthyroxinaemia in man include peripheral resistance to thyroid hormones, acute non-thyroidal illness, acute psychiatric illness and drug administration.

Peripheral resistance to thyroid hormones is an inherited condition and is associated with elevated total T3 and free T4 and

T3 concentrations. Transient hyperthyroxinaemia of acute non-thyroidal illness results from a combination of decreased protein binding and decreased extra-thyroidal T4 metabolism and shows normal to low T3 concentrations. Transient hyperthyroxinaemia of acute psychiatric illness is associated with binding abnormalities in 50 per cent of patients. The aetiology in the remainder is unknown but may be the result of amphetamine abuse or the endogenous production of an amphetamine-like substance such as beta-phenethylamine. The condition is associated with normal to low T3 concentrations. Drug-induced hyperthyroxinaemia may occur due to the administration of the contrast agents iopanoic acid and ipodate and the antiarrhythmic agent amiodarone and results in decreased total T3 concentrations.

The only recorded cause of elevated T4 and T3 concentrations in the cat is hyperthyroidism and thus the two cases described in association with apparent euthyroidism are of particular interest. It is possible that both animals may have been hyperthyroid. However, neither showed clinical abnormalities nor had palpable thyroid masses and both are alive and healthy after a follow-up period of three years. This diagnosis is particularly unlikely in case FT/NC/3/82 which had normal T4 and elevated T3 concentrations. This animal was only three and a half years old when sampled and the youngest age for a case of feline hyperthyroidism yet recorded is six years (Peterson et al., 1983b). In addition, while T3 toxicosis has been recorded in man (Sterling, Refetoff and Selenkow, 1970; Hollander, Shenkman, Mitsuma, Blum, Kastin and Anderson, 1971; Hollander, Mitsuma, Nihei, Shenkman, Burday and Blum, 1972) and in the dog (Chastain, Hill and Nichols, 1972) it has not been recorded in cats.

Of the documented causes of elevations of both T4 and T3 concentrations in euthyroid humans, the most likely cause for the case described (FT/NC/9/82) is an inherited thyroxine-binding abnormality. It is more difficult to hypothesise about case number FT/NC/3/82 as this animal had a normal T4 with an elevated T3 concentration. This finding has not been recorded in man but could result from a variant of binding protein with increased affinity for T3 and not T4 or T3 antibody formation. The cat was also involved in a road traffic accident three weeks before sampling which further complicated the situation, but it is difficult to see how this might have influenced the abnormal results. The assay of further samples from each animal would have been desirable but neither owner would agree to this.

Studies were subsequently carried out on the serum thyroid hormone binding proteins of both of these cases, together with samples taken from other animals with selected disease conditions which had abnormal T4 and T3 concentrations. These results are reported in Chapter 9.

8. INVESTIGATIONS OF THYROID HORMONE BINDING BY SERUM PROTEINS

IN NORMAL CATS

8.1 GENERAL INTRODUCTION

The strong association of thyroid hormones with macro-molecules in serum has, retrospectively, been clear from early experiments which demonstrated that human serum iodine failed to dialyse (Gley and Bourcet, 1900) or to pass on ultrafilter (Trevorrow, 1939). However, thyroxine could readily be removed from these macro-molecules by organic solvents (Trevorrow, 1939).

Early attempts to identify proteins responsible for thyroxine binding in man by iodine analyses of plasma protein fractions (Taurog and Chaikoff, 1948) implicated most of the serum proteins except gamma-globulins. The presence of a specific thyroxine binding globulin (TBG) was not appreciated until Gordon et al. (1952) used the technique of zone electrophoresis and subsequent studies identified that T₄ was also carried by TBPA, and to a lesser extent, by albumin. T₃ is bound only to TBG and albumin (Nicoloff, 1978).

There are major differences in thyroid hormone protein binding between species (Robbins and Rall, 1960). As previously discussed, the few reports on feline thyroid hormone binding are contradictory and a corresponding protein to human TBG has not been identified conclusively.

This chapter describes studies of thyroid hormone binding to serum proteins in normal cats. Section 8.2 reports on the binding of T₄ and T₃ in unfractionated feline and human sera. Sections 8.3

to 8.6 detail experiments performed to establish and validate the conditions for electrophoresis of thyroid hormone binding proteins in feline serum. Section 8.7 describes the qualitative and quantitative features of the binding of T4 and T3 to serum proteins in normal cats and compares these with the corresponding data for normal humans. Chapter 9 subsequently describes protein binding studies in animals with physiological or pathological variations of T4 and T3 concentrations.

8.2 BINDING OF THYROXINE AND TRIIODOTHYRONINE TO PROTEINS IN UNFRACTIONATED FELINE AND HUMAN SERA

Introduction

As an initial step in investigating the binding of T4 and T3 by serum proteins in the cat, the T3 serum uptake test (Section 3.7) and the T4 serum uptake test were performed on feline and human serum from healthy individuals. These tests would indicate differences in the overall binding of the labelled iodothyronines to serum proteins without distinguishing between the relative concentrations of individual proteins.

Reagents

Radiolabelled thyroxine and triiodothyronine. ^{125}I -T4 was as described in Section 5.2. ^{125}I -T3 (1 MBq/nmol to 1.4 MBq/nmol) was obtained from the same source.

Absorbent. Sephadex G-25 (fine) was obtained from Pharmacia, Milton-Keynes, Buckinghamshire, England.

Buffer. The buffer used was 0.02 mol/l sodium phosphate, pH 7.2 containing 0.2 per cent (w/v) gelatine and 0.01 per cent (w/v) sodium azide as bacteriostat. The stock solution was stored at 4°C.

Reference and quality control sera. The reference serum was Wellcontrol 1 (Wellcome Ltd., Berkhamsted, Hertfordshire, England). Quality control sera for the T3 uptake test were Riatrac 1, 2 and 3 obtained from Beckton-Dixon Immunodiagnostics, Orangeburg, New York, USA.

Equipment. All equipment was as described in Section 5.3 with the exception of:

Assay tubes. All reactions were carried out in duplicate in round-bottom polystyrene tubes of 8.5 ml capacity obtained from W. Sarstedt Ltd. (address as in Section 5.3).

Method

The reagents and samples were allowed to come to room temperature and each was mixed well before use.

Triiodothyronine uptake tests. A working solution of ^{125}I -T3 was prepared by adding 15 μl of the stock radiolabel to 75 ml buffer. The reference serum, quality controls and samples (three sera from healthy cats and three from healthy humans, the latter kindly supplied by Dr. G.J. Beckett), together with a sample of assay diluent were tested in duplicate.

Sephadex G-25 (400 mg) was delivered to each assay tube by means of a pre-calibrated scoop. 2.5 ml ^{125}I -T3 working solution was added to each assay tube using an automatic diluter. 25 μl of sample, quality control serum or reference serum was then added to the respective tubes by means of an adjustable pipette.

After mixing in a multi-tube vortex mixer for 15 minutes, the tubes were then centrifuged at room temperature for 15 minutes at 3,000 rpm. 0.5 ml of supernatant was then transferred to clean 12 x 75 mm tubes and counted in a gamma counter.

Thyroxine uptake test. A working solution of ^{125}I -T₄ was prepared by adding 60 μl ^{125}I -T₄ to 75 ml buffer.

The T₄ uptake test was performed as the T₃ uptake test, using the same samples and quality controls and buffer alone.

Results

Table 8.1 shows the number of counts per minute per 0.5 ml of supernatant fluid (i.e. bound to serum proteins) for each of the test samples, together with the group mean values and the value for buffer alone.

Triiodothyronine uptake test. The T₃ serum uptake, with a correction for the particular reference serum used, is given by the equation:

$$\text{T3 serum uptake} = \frac{\text{counts for test serum}}{\text{mean counts for reference serum}} \times \text{Value of reference serum}$$

The results for the quality controls for the T₃ uptake test were satisfactory and are presented in Table 8.2.

The mean number of counts in the supernatant was 2.3 times that of buffer for feline serum and 5.9 times that of buffer for human serum. A mean of 2.5 times the mass of radiolabel was bound by human compared with feline serum.

Thyroxine uptake tests. Values for the quality controls were not available for the T₄ uptake test.

The mean number of counts in the supernatant was 3.6 times that of buffer with feline serum and 4.9 times that of buffer for human serum. Human serum bound a mean 1.4 times that of the same volume of cat serum.

Discussion

The T₃ uptake test has been widely employed as a test of

thyroid function in man and its use with healthy cat sera has also been reported (see Section 3.7). As carried out here, the amount of radiolabelled iodothyronine in the supernatant reflects the concentrations of unsaturated thyroid hormone binding proteins, which in turn depend upon the serum T₄ and the total concentration of the thyroid hormone transport proteins.

Results of the quality controls for the T₃ uptake test were expressed relative to a reference serum so as to avoid changes that would otherwise occur if the absolute uptake were used, owing to slight changes in assay conditions and reagents. The quality control samples gave satisfactory results at all levels in the T₃ uptake test. It was not possible to evaluate quality control results for the T₄ uptake test since target values for the controls were unavailable. However, the same distribution of counts was obtained with the three control samples in both the T₄ and T₃ tests, suggesting that the results were of the correct order.

T₃ appeared to be poorly bound in cat sera compared to human sera. In this study, human serum bound a mean of 2.7 times the ¹²⁵I-T₃ than an equal volume of cat serum. Other workers who have used the T₃ uptake test in cats have reported similar results. Tanabe, Ishii and Tamaki (1969) and Kallfelz and Erali (1973) found a T₃ uptake (determined as radioactivity bound to the absorbent, and therefore the inverse of the results expressed above) which was higher than any other mammalian species studied with the exception of the mouse.

T₄ was also **more** avidly bound in human compared with cat serum, but in contrast to T₃, the difference was less marked, human serum binding only 1.4 times the ¹²⁵I-T₄ of feline serum.

It is probable that this reflects more avid binding of T4 than T3 by feline serum proteins, but confirmation of this hypothesis would require equilibrium dialysis studies to determine the relative percentages of free T4 and T3. The reduced degree of T3 and T4 binding in feline compared to human serum may be explained by reduced capacity or affinity of the binding proteins, or higher concentrations of iodothyronines by comparison with human serum. The latter possibility can be discounted as serum T4 and T3 concentrations are considerably lower in cats than in man. The present study does not give specific information about the capacity or affinity of feline binding proteins. By means of electrophoresis and autoradiography of feline serum labelled with ^{131}I -T4, Tanabe, Ishii and Tamaki (1969) found that the radioactivity of what they termed the "post-albumin" band did not disappear when increments of T4 were added. They argued that this zone could not be TBG which in man is characterised by saturability at low T4 concentrations. Scherzinger, Guzy and Lorcher (1972) made similar observations, but felt that the zone did correspond to human TBG but was of lower capacity, with most T4 being bound to albumin at normal concentrations.

The following sections of this chapter attempt to define more specifically the nature of the binding of iodothyronines in the cat by means of the fractionation by electrophoresis of serum proteins previously labelled with ^{125}I -T4 or ^{125}I -T3.

8.3 METHODOLOGICAL STUDIES OF IODOTHYRONINE BINDING TO PROTEINS IN FELINE SERUM. (i) PRELIMINARY INVESTIGATIONS USING AGAROSE GEL ELECTROPHORESIS

Introduction

Preliminary studies of serum T4 and T3 binding were carried out with a commercially available agarose gel electrophoresis system. Such gels are easily managed and preserved. Diethylbarbiturate buffer, pH 8.6 is recommended for use with this system. However, barbiturate has been shown to inhibit T4 binding to prealbumin in human plasma (Ingbar, 1958; Farer et al., 1962). In addition, Tata (1959) showed that T4 binding to human albumin varied between physiological pH and pH 8.6. In order to avoid these problems, initial studies were carried out with an alternative buffer at physiological pH.

Reagents

Radiolabelled thyroxine and triiodothyronine. ^{125}I -T4 and ^{125}I -T3 were as described in Section 5.2.

Support medium. The support medium was a commercially available agarose gel containing arabic acid, and sodium azide as a bacteriostat, and diethylbarbiturate buffer as described below (Beckman SPE Gels, Beckman Instruments Inc., Fullerton, California, USA).

Buffer. The buffer was 0.05 mol/l tris-maleate containing 0.05 mol/l tris (hydroxymethyl) methylamine and 0.05 mol/l maleic acid, the pH being adjusted to 7.4 by the addition of 1.0 mol/l sodium hydroxide. The stock solution was stored at 4°C.

Acid-alcohol solution. Washing and destaining of the gels was carried out in acid-alcohol solution comprising six parts

of 100 per cent ethyl alcohol, one part of glacial acetic acid and three parts of deionised water. This solution was stored at room temperature in a closed container.

Acetic acid solution. The fixative was five per cent aqueous acetic acid solution. The solution was stored at room temperature.

Protein stain. The protein pattern was visualised by staining with Paragon blue (disodium salt of disulphonic acid), obtained from Beckman Instruments and dissolved in five per cent aqueous acetic acid solution according to the manufacturer's instructions.

Film. Autoradiography was carried out using Fuji RX (Safety) 4 X-ray film (Fuji Photo Film Company (UK) Ltd., Cresta House, 125 Finchley Road, London, England).

Equipment

Reaction tubes. Serum samples were incubated with ^{125}I -T4 or ^{125}I -T3 in 12 mm x 75 mm glass tubes (Corning Pyrex Borosilicate Culture Tubes, Corning Glass Works, Corning, New York, 14830, USA).

Pipettes. Serum samples were applied to the gels using an automatic pipette (Beckman Paragon Applicator 656030), with disposable pipette tips.

Electrophoresis system. Electrophoresis was carried out using the Beckman Paragon Electrophoresis System comprising the Paragon Cells (number 655802) assembled into the Paragon Power Supply (number 655803). Drying took place in the Paragon Dryer (number 655805) and fixing, staining and destaining in the Paragon Wet Processor (number 655809). Electrophoresis and X-ray films were scanned by a Beckman R112 Scanning Densitometer.

Gamma counter. A Nuclear Enterprise NE 1600 was used and maintained as in Section 5.3.

X-ray film processor. X-ray films of autoradiographs were processed automatically in a Fuji X-Ray Processor, RGII at a developer temperature of 32°C and a dryer temperature of 30°C.

Methods

Thyroxine binding to serum proteins. From previous studies, a mean T4 concentration for feline serum of 30 nmol/l was assumed. It was also assumed that the same concentration of ^{125}I -T4 could be added without disturbing the equilibrium. The tracer used had a specific activity of 1nCi/pg T4 and binding of the radiolabel would be detected radiographically by a 48-hour exposure.

Gels were soaked overnight at room temperature in tris-maleate buffer to replace contained diethylbarbiturate buffer. The reagents and samples were allowed to come to room temperature and each was mixed well before use.

Twenty μl ^{125}I -T4 was added to each sample tube and the ethanol:water evaporated by nitrogen. Fifty μl of test serum was added to each tube and allowed to equilibrate for one hour at room temperature. During development of the technique, buffer replaced serum in one tube so that the position of the electrophoresed, unbound ^{125}I -T4 could be determined. Samples were vortex-mixed before, during and after equilibration.

Gels were blotted gently with the gel blotter provided. Electrophoresis runs were arranged with test samples in positions one to three inclusive, with duplicate samples containing radiolabel in positions six, eight and ten. Positions four, five, seven and nine were not used, to facilitate cutting. Five μl samples were

placed onto the gel via the sample template provided and allowed to diffuse into the gel for five minutes. Excess sample was removed by blotting with the template blotter provided and the template removed.

Gels were electrophoresed at a constant 100 volts for 25 minutes. The gel was then cut to separate the radiolabelled and non-radiolabelled parts. The non-radiolabelled part of the gel was placed in acid-alcohol solution for five minutes and then into the drying oven for a minimum of ten minutes. When completely dry, it was stained with Paragon blue solution for three to five minutes. It was then immersed sequentially for two-minute intervals in acetic acid solution, acid-alcohol solution and acetic acid solution. After drying, the films were scanned densitometrically at 600 nm to determine serum protein concentrations.

The radiolabelled part of the gel was rapidly dried by hot air, and covered in thin, transparent kitchen film to prevent the gel adhering to the X-ray film. The gel, in contact with the X-ray film, was then clamped between glass plates for 48 hours. The film was processed and the subsequent autoradiograph scanned densitometrically as above. By comparison with the stained electrophoretogram, the corresponding radiolabelled film was cut according to its protein fractions and the radioactivity of each was counted in a gamma counter.

^{125}I -T4 in buffer was electrophoresed as above and the resultant gels cut by comparison to stained electrophoretograms of normal sera and counted in a gamma counter.

Background counts were deducted from all observations prior to percentage binding evaluation.

Triiodothyronine binding to serum proteins. This was investigated as above with the following modifications: From previous studies, a mean T3 concentration for feline serum of 1 nmol/l was assumed. It was also assumed that the same concentration of ^{125}I -T3 could be added without disturbing the physiological equilibrium. The tracer used had a specific activity of 1nCi/pg T3. While this was sufficient for detecting ^{125}I -T3 distribution by cutting and gamma counting the gels, it was unlikely that suitable autoradiographs would be obtained except with very long exposures, but initially a five-day exposure was investigated. Ten μl of ^{125}I -T3 per sample was used.

Results

Initial pilot experiments using four sera from healthy cats together with two buffer studies were carried out for both T4 and T3.

Serum protein separation. This technique readily separated the serum proteins into five fractions (Fig. 8.1). From the fastest migrating component, these were: albumin, alpha-1-globulin, alpha-2-globulin, beta-globulin and gamma-globulin. The alpha and beta-2-globulins could generally be sub-divided into alpha-1a, alpha-1b, alpha-2a, alpha-2b, beta-2a and beta-2b fractions. However, such separation was not possible in all samples and the beta-1 and gamma-globulin fractions generally could not be subdivided. A prealbumin fraction was not demonstrable.

Thyroxine binding to serum proteins. Autoradiographs showed two bands of radioactivity in all sera tested. When compared to the stained electrophoretogram, the first band corresponded to the albumin zone. The second band began at the leading edge of the

alpha-1-globulin and blurred into the alpha-2-globulin area (Fig. 8.1).

Autoradiographs of the radiolabel electrophoresed in buffer only showed non-protein bound T4 to be distributed towards the trailing edge of the alpha-1-globulin extending into the alpha-2-globulin, i.e. it had slower mobility than the non-albumin bound label in serum (Fig. 8.2).

Superimposition of the densitometer scans of the electrophoretograms and the autoradiographs confirmed qualitatively the distribution of the radiolabel in the serum samples (Fig. 8.3).

The results of quantitative assessment of ^{125}I -T4 distribution in normal feline sera and unbound in buffer are presented in Table 8.3. There was good agreement between the cutting and counting and scanning densitometry techniques with no significant difference being demonstrated by Student's paired t test ($t=0.05$, 24 d.f; $P>0.9$). The major part of the ^{125}I -T4 was present in the albumin and alpha-1-globulin fractions with a smaller percentage in the alpha-2-globulin fraction. Negligible amounts were present in the beta and gamma-globulin fractions.

The distribution of radiolabel in the two samples of ^{125}I -T4 electrophoresed in buffer only showed good agreement. Here, only a small percentage of the radiolabel was carried in the position corresponding to albumin. The major carriage was in a position corresponding to the alpha-globulin zone although the percentage distribution in the alpha-1 and alpha-2 components was marginally decreased and markedly increased respectively by comparison with electrophoresed serum samples.

Triiodothyronine binding to serum proteins. Auto-radiographs were not obtained after a five-day film exposure and ^{125}I -T₃ distribution was therefore evaluated by cutting and counting the gels by comparison with the corresponding stained electrophoretogram.

The results of this study are shown in Table 8.4. Apart from the albumin zone where negligible amounts of radioactivity were present, ^{125}I -T₃ was demonstrated in the four other main protein fractions, the major percentages being in the alpha-2 and the beta-2-globulin zones. Relatively small percentages were found in the alpha-1 and the gamma-globulin fractions. This distribution of radiolabel was clearly different from that in buffer where the majority of the radioactivity was present in the gamma-globulin fraction.

Discussion

The principles of electrophoresis are based on the fact that a charged particle placed in an electric field will migrate towards the oppositely charged electrode at a rate depending upon the electrical charge of the particle, the strength of the electrical field and the nature of the support medium.

Amino acids and therefore proteins themselves are amphoteric, that is, they exist as positive ions in acidic solution and negative ions in basic solutions. Both amino acids and proteins possess an isoelectric point, the pH value at which the number of positive and negative charges on the molecule balance each other. In a solution with a pH of its isoelectric point, a protein has a neutral charge and does not migrate if a direct current potential is applied to the solution. The higher the pH value above the

isoelectric point, the greater is the negative charge, and the farther the protein will migrate towards the positive electrode when placed in an electric field. Conversely, the lower the pH value below the isoelectric point, the greater will be the positive charge, and the farther the protein will migrate toward the negative electrode when electrophoresed. Agarose gel electrophoresis, as used in this study, separates proteins according to charge. Some other support media separate molecules according to size (discussed later).

The characteristics of the buffer also affect the migration of proteins during electrophoresis. This is discussed under Section 8.4.

After consideration of these factors, 0.05 mol/l tris-maleate buffer, pH 7.4 was selected for initial experiments and produced good separation of protein zones.

The study is based on the principle that exogenous and endogenous hormone are bound and behave similarly. This clearly can only be the case if the amounts of radiolabel added are not excessive. Serum proteins which bind iodothyronines at physiological concentrations are not saturated at these concentrations. Therefore, a mass of radiolabel must be used which is sufficient to act as a marker during electrophoresis but which does not displace the normal physiological equilibrium. In these studies, a mass sufficient approximately to double the normal mean hormone concentration was used.

General conclusions. These preliminary studies established the following important methodological points:

(i) Feline serum proteins can be satisfactorily separated by agarose gel electrophoresis at pH 7.4 in tris-maleate buffer.

(ii) The distribution of iodothyronine binding can be demonstrated by prior radiolabelling.

In the case of T₄, this revealed the presence of binding in two zones - albumin and alpha-1/alpha-2-globulin. Unfortunately, T₄ in buffer only, also migrated in the more cathodal part of the latter zone. Although the high percentage of label in serum in this zone, and the slight difference in its mobility from free T₄ suggested that it was indeed protein bound, Farer, Robbins and Blumberg (1962) using paper electrophoresis with a number of buffers, found that T₄ could migrate as the unbound hormone when added to serum in which T₄ binding proteins were weak or lacking. Consequently, further experiments (described in Sections 8.4 to 8.6) were performed to try to confirm the presence of T₄-binding to this globulin fraction.

In the case of T₃, there was apparent binding in the alpha-1, alpha-2, beta and gamma-globulin fractions. This wide distribution of binding is not seen in human serum and in order to examine whether it was a feature of the particular electrophoresis system selected, further experiments (described in Section 8.4) were performed. T₃ in buffer only migrated in significant quantities in the area corresponding to gamma-globulin.

(iii) Autoradiography provided a convenient technique for visualising the thyroid hormone binding proteins for T₄ but not for T₃. This arose from constraints on the amount of labelled iodothyronine that could be added without disturbing the equilibrium.

(iv) Quantitative estimation of thyroid hormone binding by the different proteins could be carried out by cutting and gamma counting the zones or by scanning the autoradiograph

densitometrically. The agreement between these techniques was excellent. Although scanning the autoradiograph was more convenient and less time-consuming, the cutting and counting technique offered continuity between the T4 and T3 studies.

8.4 METHODOLOGICAL STUDIES OF IODOTHYRONINE BINDING TO PROTEINS IN FELINE SERUM. (ii) EFFECT OF pH CHANGES AND BUFFER TYPE ON ELECTROPHORESIS USING AGAROSE GEL

Introduction

In an attempt to resolve the bands corresponding to the globulin bound and unbound T4 and whether or not multiple zone binding of T3 was an artefact of the electrophoresis system, the effects of varying the buffer type and pH were investigated.

Reagents and equipment

These were as described in Section 8.3, with the exception of an additional buffer. This was 0.1 mol/l diethylbarbiturate, pH 8.6 (Beckman B-2 buffer, Beckman Instruments). The prepared buffer was stable for 60 days at room temperature.

Methods

Effect of changing pH of tris-maleate buffer on

thyroxine binding to serum proteins. 0.05 mol/l tris-maleate buffer was prepared as described previously. The pH of aliquots of the buffer was adjusted to 6.8, 7.0, 7.2 and 7.4 by the dropwise addition of 1.0 mol/l sodium hydroxide. Sera from two healthy cats and samples of buffer of each pH were incubated with ^{125}I -T4 and subsequently electrophoresed at all four pHs by the method described previously. The radiolabelled gels were then autoradiographed for 48 hours.

Effect of diethylbarbiturate buffer on thyroxine and

triiodothyronine binding to serum proteins.

Sera from two normal humans (kindly supplied by Dr. G.J. Beckett) and from three healthy cats were used in these studies. Samples were radiolabelled with the respective iodothyronines and electrophoresed on agarose gel as described in Section 8.3 but using 0.1 mol/l diethylbarbiturate buffer, pH 8.6. In each case, the corresponding radiolabelled iodothyronine in buffer alone was also electrophoresed on the same gel. The resulting gels were processed as described previously with the exception that none of the studies was autoradiographed.

Results

Effect of changing pH of tris-maleate buffer on

thyroxine binding to serum proteins.

The resultant autoradiographs are shown in Fig. 8.4. Sequential reductions in pH from physiological failed to change the position of the T4 in buffer relative to that in serum. Cutting and gamma counting of the gels was not, therefore, carried out.

Effect of diethylbarbiturate buffer on thyroxine and

triiodothyronine binding to serum proteins

Serum protein separation.

Typical stained electrophoretograms of normal human and feline serum run in parallel are shown in Fig. 8.5.

The proteins in feline serum were more mobile than those in human serum, resulting in electrophoretograms which were ten per cent longer with cat serum. There was little difference in the definition of the protein bands.

Prealbumin fractions were not demonstrated with either human or feline sera by this technique.

Thyroxine binding to serum proteins. The results of this study are presented in Table 8.5.

With feline serum, approximately equal amounts of ^{125}I -T₄ were bound in albumin and alpha-1-globulin. Negligible amounts of radiolabel were found in the three other fractions. Unfortunately, an almost identical distribution was found when the radiolabel was electrophoresed free in buffer and the resultant gel cut according to a stained feline serum electrophoretogram.

The percentages of ^{125}I -T₄ were similar in human and feline alpha-2-globulin, beta-globulin and gamma-globulin. However, much higher amounts of radiolabel were found in the alpha-1-globulin fraction in man than in the cat and there was a corresponding reduction in the albumin zone. When ^{125}I -T₄ electrophoresed free in buffer was cut according to a stained human serum protein gel, almost all of the radioactivity was found in the area corresponding to the albumin zone, with only a small percentage being found in the other four areas.

Triiodothyronine binding to serum proteins. The results of this study are presented in Table 8.6.

The pattern of protein binding was similar in both feline and human sera with the major proportion being in the alpha-globulin zone. Approximately twice the percentage of ^{125}I -T₃ was bound by human as by feline albumin. Significant amounts of radiolabel were found in the beta-globulin in both species. The majority of ^{125}I -T₃ electrophoresed free in buffer travelled in areas corresponding to the alpha-2-globulin zone of both feline and human serum. Significant amounts were also found in the area corresponding to alpha-1-globulin of human serum.

Discussion

The characteristics of a buffer which affect the migration of proteins during electrophoresis are its type, concentration, ionic strength and pH.

The effects of buffer concentration arise because current is carried by ions present in solution. The greater the number of buffer ions present relative to other ions, the greater will be the proportion of current they carry. The other ions will move more slowly but this results in sharper definition of the protein fractions.

As buffer ionic strength increases, heating within the system increases although the resolution of the fractions is enhanced (Smith, 1968). In practice, ionic strengths between 0.01 mol/l and 0.10 mol/l are commonly used.

Changes in buffer pH affect the distances travelled by charged molecules during electrophoresis. Different molecules carrying different amounts of charge should be affected to varying degrees as described in Section 8.3.

Effect of changing the pH of tris-maleate buffer on

thyroxine binding to serum proteins. In this study, it was hoped that a reduction in buffer pH would retard the travel of free T4 more than that which was protein bound. The reasons why this did not occur are not immediately obvious. It is possible that the travel of the protein fractions and the free T4 were retarded to the same degree, with the latter remaining in the same relative position to the alpha-1 and alpha-2-globulin fractions. Indeed, the resultant autoradiographs and those shown in Fig. 8.2 can be virtually superimposed suggesting that this is the case. It may be that the

free T4 and protein fractions could have been variably retarded if the pH of the buffer had been reduced further. However Tata (1959) found that there was an eight per cent increase in binding of T4 by human TBG between pH 7.4 and 8.6. Such studies with cat serum have not been carried out but it was felt that further reductions in pH would create an unacceptable deviation from physiological conditions.

Effect of diethylbarbiturate buffer on thyroxine and triiodothyronine binding to serum proteins. Barbiturate buffer, pH 8.6 has been the favoured buffer for studies on T4 and T3 binding in human serum. The inclusion of samples of human serum in this study, therefore, allowed comparison of the results with those published by other workers.

Barbital buffers are monovalent and therefore they contribute to the ionic strength to a lesser degree than polyvalent ions. Ions of low valency interact minimally with proteins. They are also very large and thus migrate slowly in the electrical field so that the conductivity and, therefore, the Joule heat effect can be kept to a minimum. However, pH 8.6 is not physiological and barbiturate buffer blocks the binding of T4 to TBPA (Ingbar, 1958; Farer et al., 1962).

There was little difference in the distribution of T4 in cat serum in either barbiturate or tris-maleate buffer with the majority of radiolabel being present in albumin and alpha-1-globulin. Negligible amounts were found in the remaining protein fractions. Similar findings using barbiturate buffer have been reported by Robbins, Apter and Rall (1957), Robbins and Rall (1960), Farer et al. (1962) and Scherzinger, Guzy and Lorcher (1972). In

this study, unfortunately, an almost identical distribution occurred when the radiolabel was electrophoresed in buffer alone, making it impossible to differentiate between bound and free T4 in serum.

In contrast, there were marked differences in the distribution of T3 in feline serum electrophoresed in tris-maleate and barbiturate. Such differences due to buffer type have been well documented in work with human serum. Without studies utilising alternative techniques, it was not possible to decide which was reflecting the situation in vivo.

Comparing the tracer distributions in feline and human sera in barbiturate buffer, much higher percentages of T4 were found in the alpha-1-globulin region of human than in feline serum with resultant inverse findings with albumin. Because ^{125}I -T4 electrophoresed in barbiturate alone travels at a point corresponding to albumin, it was possible to confirm that human alpha-1-globulin bound T4 in this system.

The pattern of T3 distribution in human and feline serum with barbiturate buffer was similar. When electrophoresed free in buffer, T3 travelled at a point corresponding to alpha-2-globulin in both. Thus, it is only possible to report that both feline and human albumin and alpha-1-globulins bind T3 in barbiturate.

Because there was less overlap between T3 electrophoresed in serum and free in buffer, with tris-maleate than with barbiturate, and because the tris-maleate was at physiological pH, further studies were carried out with this buffer.

Unfortunately, this technique failed to separate TBPA from sera of either species. TBPA does not appear to have been described in the cat and it is therefore impossible to speculate whether

this is a failure of the present technique or whether the protein is lacking in this species. Tata (1959) suggested that in certain buffer systems, TBPA was dissociated from an alpha-globulin and that in barbiturate, TBPA migrated electrophoretically as an inter-alpha-globulin. Other data, however, have been more easily explained on the basis that TBPA is prevented from binding T4 in barbiturate by some sort of competitive process (Robbins and Rall, 1960). It is interesting to speculate that, in the present studies with human serum, TBPA may have migrated with alpha-globulin. If such were the case, the results of the present study agree closely with figures compiled from the literature for man by De Groot and Stanbury (1975).

8.5 METHODOLOGICAL STUDIES OF IODOTHYRONINE BINDING TO PROTEINS IN FELINE SERUM. (iii) RADIOIMMUNOELECTROPHORESIS

Introduction

In view of the difficulties in distinguishing reliably between globulin bound and free T4 by the original technique and by alterations in the buffer pH, radioimmuno-electrophoresis was carried out to attempt to demonstrate the specific T4 binding proteins in feline serum.

Immuno-electrophoresis was first described by Grabar and Williams in 1953 and was modified to a micro technique by Scheidegger (1955). The method is a combination of two techniques, gel diffusion and immunodiffusion. It is capable of differentiating the physicochemical and antigenic properties of proteins. Radioimmuno-electrophoresis is a further modification of the procedure in which specific protein fractions are made visible autoradiographically after prior radiolabelling.

Reagents

Support medium. The support medium was a commercially available agarose gel (Beckman IEP Gels), containing diethylbarbiturate as described below.

Buffer. The buffer was 0.1 mol/l diethylbarbiturate, pH 8.6 (Beckman B-2 buffer) as described previously.

Saline solution. 0.85 per cent saline solution was prepared from reagent grade sodium chloride and deionised water.

Anti-cat serum. Goat anti-cat serum containing 0.1 per cent sodium azide as preservative was supplied in liquid phase by Miles-Yeda, Miles Laboratories, Stoke Poges, England. It was stored in aliquots at -20°C.

Other reagents. All other reagents were as described in Section 8.3.

Equipment

All equipment was as described in Section 8.3.

Method

The reagents and samples were allowed to come to room temperature and each was mixed well before use.

Samples of serum from two healthy cats were incubated with ^{125}I -T4 for one hour as described in Section 8.3. Gels were blotted gently with the gel blotter provided. Each gel could take seven test samples. Electrophoresis runs were arranged with two aliquots of the sample under test in lanes five and seven with duplicate samples containing radiolabel in positions two and three. Position four was purposely left free to facilitate cutting the gel.

Five μl samples were placed onto the gel via the sample template provided and allowed to diffuse into the gel for ten

minutes. Excess sample was removed by blotting with the template blotter provided and the template removed.

Gels were electrophoresed for 20 minutes at a constant 100 volts. After removing the gel from the cell, the electrophoresed samples were separated by cutting. One radiolabelled and one unlabelled sample were then immunodiffused. Twenty μ l of goat anti-cat serum was applied to the antiserum troughs via the antiserum template provided. After ten minutes, the antiserum template was removed and the gel placed in a covered incubation tray for 24 hours on a gel blotter moistened with deionised water.

The remaining electrophoresed samples were immediately dried at up to 90°C for five minutes. The unlabelled sample was then stained as described in Section 8.3. The labelled sample was covered in kitchen film and retained for 24 hours.

After 24 hours' immunodiffusion, the unlabelled sample was stained as described in Section 8.3.

In the initial study, the radiolabelled, diffused sample was soaked in saline for ten minutes and press-dried on blotting paper, each on two occasions, and subsequently dried completely in the drying oven, as per the manufacturer's instructions. This procedure was found to cause dissociation of the bound T4 and subsequently the saline washing and the press-drying were omitted.

After covering with kitchen film, the labelled, diffused sample and the labelled, non-diffused sample were autoradiographed for 48 hours as described in Section 8.3.

Results

The results of this study are presented in Fig. 8.6.

The stained electrophoretogram has a rocket-like appearance with the leading edge of the albumin being pointed. The albumin and

gamma-globulin zones are well-defined but other zones are more difficult to distinguish. The corresponding autoradiograph shows radiolabel present in the albumin zone moving back into the alpha-globulin zone.

The immunoelectrophoresed sample shows seven areas, the leading and most prominent one being albumin and the trailing one being gamma-globulin. The corresponding autoradiograph shows a wide, rocket-shaped distribution of radioactivity. There was no evidence of areas corresponding to either albumin or any specific globulin binding T4.

Discussion

The principle of immunoelectrophoresis is based upon the visualisation of specific proteins through the formation of antigen-antibody precipitin lines following protein separation by electrophoresis. The simultaneous diffusion of the separated proteins in the sample and the antibody in the trough results in the formation of precipitin areas, the shape and position of each being determined by the nature and concentration of each protein present. The areas represent the positions in the gel at which equivalence between antigen and antibody concentrations exist. If a control sample is available, direct comparisons of the precipitin areas can be made.

If the protein sample is mixed with a radiolabel prior to electrophoresis, any protein binding the radiolabel may be demonstrated by autoradiography after the immunodiffusion phase.

In this study, excellent separation and visualisation of the protein zones were achieved both by simple electrophoresis and by immunoelectrophoresis, although identification of specific

constituent proteins was not possible because of the non-availability of feline serum protein controls. Radioimmuno-electrophoresis was unsatisfactory. When the manufacturer's instructions with regard to saline washing and press-drying were followed, autoradiographs were not produced. This may have resulted from total dissociation of ^{125}I -T4 from its binding proteins or ^{125}I -T4 may have remained in the precipitin areas but in insufficient amounts for autoradiography. The latter explanation is probably correct, as when Bigler (1976a) used a similar technique, exposure times of eight weeks were required to give satisfactory autoradiographs.

When the washing and press-drying stage was omitted, ^{125}I -T4 remained bound to proteins diffusely over the area of electrophoresis. This may have been due to non-specific binding of ^{125}I -T4 to the anti-feline serum, which would normally have been removed by the washing step.

Radioimmuno-electrophoresis has been successfully applied to the visualisation of antigen-antibody reactions but was unsatisfactory for the demonstration of T4 binding. This may have been due to insufficient sensitivity of detection of the label, or the relatively reversible nature of the TBG-T4 binding compared with that of antigen-antibody binding, or a combination of these factors. This experiment did not exclude the presence of a T4 binding globulin and an alternative approach was adopted to investigate its presence.

8.6 METHODOLOGICAL STUDIES OF IODOTHYRONINE BINDING TO PROTEINS

IN FELINE SERUM. (iv) USE OF POLYACRYLAMIDE GEL

ELECTROPHORESIS

Introduction

Electrophoresis using agarose gel separates molecules mainly according to charge. Support media such as polyacrylamide gel act as molecular sieves, with small molecules progressing rapidly and larger molecules more slowly. T₄ is a relatively small molecule by comparison with serum proteins. When ¹²⁵I-T₄ in buffer alone is electrophoresed in parallel with ¹²⁵I-T₄ in serum, the radiolabel in the buffer should move more rapidly than the albumin and is likely to pass completely through the gel during protein separation.

The aim of this study was to demonstrate ¹²⁵I-T₄ binding in the albumin and alpha-globulin regions of normal feline serum electrophoresed on polyacrylamide gel, while showing that the radiolabel was not present in corresponding areas of the gel when electrophoresed in buffer alone.

Reagents

Radiolabelled thyroxine. ¹²⁵I-T₄ was as described in Section 5.2.

Acrylamide. Acrylamide used in the production of the stacking and resolving gels was obtained from BDH Chemicals Ltd., Poole, Dorset, England.

Temed. The compound used to accelerate polymerisation was N, N, N¹, N¹ - tetramethylethylenediamine obtained as Temed from BDH Chemicals Ltd.

Buffers. Three buffers were used:

(i) 0.375 mol/l tris-hydrochloride buffer, pH 6.8 was used to prepare the stacking gel.

(ii) 1.0 mol/l tris-hydrochloride buffer, pH 8.85 was used to prepare the resolving gel.

(iii) The electrode buffer was 0.05 mol/l tris-maleate as described in Section 8.3.

All buffers were stored at 4°C.

Fixing solution. The electrophoresed proteins were fixed using a solution containing 57.5g trichloroacetic acid and 17.25g sulphosalicylic acid in 500 ml distilled water.

Protein stain. The protein stain was 0.2 per cent Coomassie brilliant blue R in methyl alcohol: water: acetic acid (50:50:7 by volume). The stain was stored at room temperature.

Acid-alcohol solution. Washing and destaining of the gels was carried out using a solution of methyl alcohol: acetic acid: distilled water (5:7:88 by volume).

Film. Fuji RX (Safety) 4 X-ray film, as described in Section 8.3, was used for autoradiography.

Equipment

Electrophoresis system. Electrophoresis was carried out using an LKB 2001 vertical electrophoresis unit obtained from LKB-Wallac, Turku 10, Finland.

Other equipment. Additional equipment was as specified in Section 8.3.

Methods

All reagents and samples were allowed to come to room temperature and each was mixed well before use.

Preparation of stock acrylamide solution. A 14 per cent solution of acrylamide was prepared by dissolving 26.6g acrylamide and 1.4g N-methylene bis-acrylamide in 200 ml distilled water. The solution was stored at 4°C.

Preparation of resolving gel. An 8.7 per cent resolving gel was prepared from 23 ml of the stock acrylamide, 30 ml of 1 mol/l tris-hydrochloride buffer, pH 8.85, 26 ml distilled water, 0.1 ml Temed and 0.1 ml freshly prepared ten per cent aqueous ammonium persulphate solution.

While still liquid, the gel was poured into the electrophoresis apparatus and a small amount of 100 per cent butanol was added to produce a sharp surface. The gel was then allowed to set and the top surface subsequently washed with distilled water.

Preparation of stacking gel. A 3.6 per cent stacking gel was prepared from 3.6 ml of the stock acrylamide, 10.0 ml of 0.375 mol/l tris-hydrochloride buffer, pH 6.8, 16 ml distilled water, 0.1 ml Temed, and 0.1 ml freshly prepared ten per cent (w/v) aqueous ammonium persulphate solution.

The stacking gel was added to the apparatus and the plastic dividing inserts introduced. After allowing the gel to set, the surface was washed with distilled water and the loose polymer removed with a pasteur pipette.

Preparation of labelled sera and buffer. Samples (50 μ l) of sera from three healthy cats and electrode buffer were incubated with 20 ml ^{125}I -T₄ for one hour as described in detail in Section 8.3. Subsequently, 10 μ l of glycerol was mixed with the labelled samples and with 50 μ l of the corresponding unlabelled sera. The glycerol caused the test samples to sink in the gel wells.

Electrophoresis. Ten μ l of each sample was added to the wells using a glass micropipette, and 10 μ l of Coumassie blue was added to one well to act as a marker. Gels were electrophoresed at a constant 100 mA until the marker dye had traversed the stacking gel. The current was then altered to 35 mA until the marker reached the bottom of the gel. The gel was then removed from the apparatus and divided through the position of the marker dye.

The unlabelled gels were placed in fixer for 45 minutes and then in acid-alcohol solution for five minutes. After staining with Coumassie blue for ten minutes, the gel was destained with several changes of acid-alcohol solution until the background was clear. Gels were stored in the short-term in the acid-alcohol solution.

The labelled part of the gel was placed in a polythene bag in direct contact with, but not clamped to, the X-ray film and autoradiographed for 48 hours.

Results

Qualitative results of this study are shown in Fig. 8.7.

Serum protein separation is more complete than with the agarose gel technique, with a number of protein components being demonstrable. The corresponding autoradiograph shows two zones of radiolabel in the serum samples corresponding to albumin and the alpha-globulin fractions. The labelled buffer sample fails to show a zone of radioactivity autoradiographically, indicating complete passage of the ^{125}I -T4 through the gel.

Discussion

Polyacrylamide gel results from the polymerisation of acrylamide monomer into long chains with cross-linking. The

polymerisation is initiated by ammonium persulphate and accelerated by Temed. The effective pore size of polyacrylamide gels is greatly influenced by the acrylamide concentration in the polymerisation mixture, pore size decreasing as acrylamide concentration increases. Gels with acrylamide concentrations of less than 2.5 per cent sieve large molecules but are almost fluid, whereas those of more than 30 per cent will sieve molecular weights of only a few thousand. A discontinuous or multiphase system (as employed here) employs a large bore stacking gel, which concentrates the proteins into extremely narrow zones prior to migration through the smaller bore resolving gel. This aids the separation of proteins during electrophoresis and their subsequent definition after staining.

In this study, a discontinuous system with a 3.6 per cent stacking gel and an 8.7 per cent resolving gel gave excellent protein separation and demonstration of the fractions binding the ^{125}I -T₄. This early success was perhaps somewhat fortuitous because, for the separation of any mixture of proteins, the choice of acrylamide concentrations is critical.

Detailed accounts of polyacrylamide gel electrophoresis of proteins in normal feline serum do not appear to have been described previously. Thus, all the protein zones shown in the stained electrophoretogram in Fig. 8.7 cannot be positively identified.

General conclusions. This study clearly established that:

(i) Feline serum proteins can be satisfactorily separated by polyacrylamide gel electrophoresis at pH 7.4 in tris-maleate buffer.

(ii) The presence of ^{125}I -T₄ in buffer alone cannot be demonstrated autoradiographically, indicating that it has passed through the gel during electrophoresis.

(iii) ^{125}I -T₄ can be identified autoradiographically in two distinct areas when electrophoresed in feline serum. These result from binding of the radiolabel to albumin and a protein with an alpha-globulin mobility.

These data confirm the results of the preliminary electrophoresis studies using agarose gel (Section 8.3) and the early reports of Farer et al. (1962) and Scherzinger, Guzy and Lorcher (1972) using other electrophoresis systems (Section 3.6).

Although polyacrylamide gel electrophoresis gave a clearer separation of feline proteins than agarose gel, it proved too complex for routine use and was not amenable to quantitative work. Agarose gel was therefore used in subsequent investigations.

8.7 BINDING OF THYROXINE AND TRIIODOTHYRONINE TO SERUM PROTEINS IN HEALTHY CATS AND HUMANS - POPULATION STUDIES USING AGAROSE GEL ELECTROPHORESIS

Introduction

The studies of serum T₄ binding by polyacrylamide gel electrophoresis strongly suggest that ^{125}I -T₄ found in the alpha-1/alpha-2-globulin protein zones in the agarose gel system is protein bound. The agarose gel system is also very satisfactory for electrophoresis of labelled T₃ in serum, as the mobility of protein bound and free T₃ differ substantially in this medium. It was possible, therefore, to apply this technique, previously used in a pilot study with four feline sera (Section 8.3), to serum T₄ and T₃ binding in a larger number of healthy cats. Results were compared with corresponding data for humans.

Methods

Serum samples from a total of 21 healthy cats (eight males, seven females, two neutered males and four neutered females) aged between five months and 11 years were selected randomly from those previously used to determine the reference ranges for T4 and T3. T4 binding studies were carried out on each sample and 20 of the samples were used in the T3 studies. T4 and T3 binding was also investigated in serum samples from two healthy humans.

Total serum protein concentrations were determined as described in Section 4.4. Agarose gel electrophoresis was carried out as described in detail in Section 8.3. With feline sera, ^{125}I -T4 binding was determined by autoradiography, by gamma counting of the five major protein zones defined by comparison with the stained, unlabelled electrophoretograms, and by densitometrically scanning the autoradiographs. With human sera, quantitation was by gamma counting of cut gel fractions only.

T3 binding was assessed similarly, with the exception that autoradiography was not used.

Results

Serum protein separation. Typical stained electrophoretograms of normal feline and human serum run in parallel are shown in Fig. 8.8.

The feline serum proteins were more mobile than their human counterparts, resulting in electrophoretograms that were 25 per cent longer with cat serum. However the protein bands were more sharply defined in the human samples. Prealbumin zones were not demonstrated with serum from either species by this technique.

The serum protein concentrations of the 21 feline samples are shown in Table 8.7.

The majority of individual values fell within the range published from this School by Keay (1982) using a different commercially available agarose gel kit with diethylbarbiturate buffer. Several values beyond the reference limits were noted, particularly with gamma-globulin concentrations (ten cases), which were also reflected in abnormal total serum protein concentrations.

Serum protein concentrations were not determined for the human samples.

Thyroxine binding to feline serum proteins. In all cases, autoradiographs were similar and showed two distinct areas of binding as shown in Fig. 8.1. The most anodal of these corresponded to the albumin fraction. The leading edge of the second zone corresponded to that of the alpha-1-globulin fraction and this zone continued cathodally, merging with the leading edge of the alpha-2-globulin fraction.

There was no significant difference between the two quantitative methods of assessment of ^{125}I -T₄ binding as assessed by Student's paired t test ($t = 0.03, 104 \text{ d.f.}; P > 0.9$) and only the values obtained by gamma counting of the gel fractions are presented (Tables 8.8 and 8.9).

The majority of the sera showed higher binding in the alpha-1-globulin than in the albumin fraction with only three showing the reverse trend. When the percentage bound in the alpha-1 and alpha-2-globulin fractions were taken together, only one sample showed higher binding in the albumin fraction. Negligible amounts of radiolabel were present in the beta and gamma-globulin fractions.

Possible variations in binding according to sex were investigated by the Mann-Whitney U-Test. Comparing females and neutered females as one group with males and neutered males as the other group, no significant difference could be demonstrated for any protein fraction. The data failed to establish any relationship between percentage T4 binding and age.

Thyroxine binding to human serum proteins. The results of this study, together with the mean results of the feline study, for comparison purposes, are presented in Table 8.10.

In human sera, a much higher percentage of ^{125}I -T4 is bound in the alpha-1/alpha-2-globulin zone than in feline sera, with inverse relationships for albumin binding. Negligible amounts of radiolabel are found in the beta and gamma-globulin fractions.

Triiodothyronine binding to feline serum proteins. The results of this study are presented in Tables 8.11 and 8.12.

There is minimal binding in the albumin fraction of all sera. The four other serum fractions, in particular the alpha-2 and beta-globulin zones appeared to bind significant quantities of T3.

Possible sex-related variations in binding were investigated by the Mann-Whitney U-Test. Comparing females and neutered females as one group with males and neutered males as the other group, no significant difference could be demonstrated for any protein fraction. The data failed to establish any relationship between percentage T3 binding and age.

Triiodothyronine binding to human serum proteins. The results of this study, together with the mean results of the feline study for comparison purposes, are presented in Table 8.13.

The majority of the radiolabel was carried in the alpha-globulin zone with a slightly higher percentage being present in the alpha-1-globulin fraction. By contrast, in the cat, the majority of the label migrated in the alpha-2 and beta-globulin zones with a small amount in the gamma-globulin. A mean of 22 per cent T3 was found in the beta-globulin in humans. A small amount of T3 was bound to human albumin but there was a negligible amount in the gamma-globulin fraction.

Discussion

Agarose gel electrophoresis was selected as the simplest technique to study T4 and T3 protein binding because kits are commercially available and the gels are simply managed, autoradiographed and preserved. Unfortunately, TBPA was not demonstrated in either human or feline sera by this method. With human serum, this is presumably a feature of the support medium, since Ingbar (1958) was able to demonstrate TBPA using paper electrophoresis in tris-maleate buffer, although at pH 8.6 rather than 7.4. The failure to demonstrate a feline TBPA may be due to technique or because the protein does not occur in feline serum.

Prior to using the technique on a larger scale, it was necessary to confirm that ^{125}I -T4 in the alpha-globulin zone was protein bound. This was achieved using the polyacrylamide gel system. Further evidence for this was obtained in later studies (see Section 9.3).

Although estimates vary according to technique, under normal physiological conditions human TBG (an inter-alpha-1/alpha-2-globulin) binds approximately 75 per cent of T4. The remainder is bound to TBPA (15 per cent) and albumin (10 per cent) (De Groot and

Stanbury, 1975). Other serum proteins may be involved in T4 transport but they carry very small amounts of the hormone (Marshall and Levy, 1966).

All published studies agree that feline albumin binds T4. However, there has been considerable debate over whether a T4 binding globulin exists in the cat, with Farer et al. (1962) and Scherzinger, Guzy and Lorcher (1972) reporting a T4 binding alpha-globulin, Tanabe, Ishii and Tamaki (1969) suggesting that this zone was really post-albumin and Bigler (1976a) failing to identify a TBG at all.

T3 binding in human serum has received less attention than T4. Using paper electrophoresis with glycine acetate buffer, pH 8.6, 65 to 70 per cent of T3 was bound to TBG and the remainder to albumin (Woeber, 1971). Investigations into feline T3 serum protein binding have not been previously reported.

These studies clearly demonstrated the existence of a T4 binding globulin in all normal cat sera studied. Peak binding occurred in the mid alpha-1-globulin zone and continued into the leading edge of the alpha-2-globulin. Human TBG migrated in an identical position in this system. However, although feline TBG bound a lower percentage (mean 59 per cent) of T4 than human TBG (in this study, mean 90 per cent), these data clearly do not support the conclusions of Scherzinger, Guzy and Lorcher (1972), who suggested that feline TBG had only a low capacity with most T4 being bound to albumin at normal concentrations.

In the feline study, only a small percentage of T3 was bound to albumin, approximately ten per cent to alpha-1-globulin, with the majority being bound by the alpha-2 and beta-globulin fractions. It is not possible, from these data, to decide whether the carriage

in the alpha-2 and beta-globulin regions represents a single or multiple protein because autoradiographic studies could not be carried out using physiological concentrations of radiolabel. Because ^{125}I -T3 electrophoresed in buffer alone ran in an area corresponding to gamma-globulin, it was not possible to deduce whether T3 in this zone in serum was protein bound or free.

Although the albumin binding of T3 in feline and human serum was similar, a much higher proportion was bound to alpha-globulin in man, due to increased alpha-1 carriage. A significant proportion was also present in the beta-globulin fraction. The significance of this observation is unknown.

In man, the concentration of the serum iodothyronine binding proteins is essentially unaffected by age, although a small but significant fall in TBPA, and increase in TBG concentrations, have been reported in the elderly (Gregerman and Davis, 1978). No age-related changes in T4 and T3 binding were noted in the present study but only two cats were older than 9 years.

General conclusions. The following general conclusions may be summarised from this study:

(i) Feline T4 is bound to albumin and a protein with a mobility in the alpha-1-/alpha-2-globulin zone.

(ii) Feline T3 is bound mainly in the alpha-2 and beta-globulin zones, with smaller proportions being present in albumin and alpha-1-globulin. It is not possible to decide whether T3 in the gamma-globulin zone is protein bound.

(iii) There are major differences in T4 and T3 binding between cats and humans. While feline and human TBG migrate in an identical position, feline TBG must be of lower affinity or capacity, or present in smaller concentrations, than that of man.

9. INVESTIGATIONS OF THYROID HORMONE BINDING BY SERUM PROTEINS IN CATS WITH ABNORMAL TOTAL THYROXINE AND TRIIODOTHYRONINE CONCENTRATIONS

9.1 GENERAL INTRODUCTION

The investigation of iodothyronine-protein interactions in serum is of interest because:

(i) variations in binding of thyroid hormones by serum proteins may explain some of the features of abnormal thyroid function, and

(ii) comparison of thyroid hormone binding in animals with and without thyroid disease may give some insight into the physiological roles of the various thyroid hormone binding proteins.

Extensive investigations have been carried out in this field in man but no such studies have been reported in cats. This chapter presents information on feline thyroid hormone protein binding in naturally-occurring hyperthyroidism, experimentally-induced hypothyroidism, and in apparent euthyroidism associated with elevated or reduced concentrations of iodothyronines. In addition, investigations were carried out into the T₄ uptake test in naturally-occurring hyperthyroidism and experimentally-induced hypothyroidism in cats, and the role of serum free fatty acid concentrations in alterations of T₄ binding in feline thyrotoxicosis.

9.2 REAGENTS AND EQUIPMENT

Thyroxine and triiodothyronine uptake tests

The reagents and equipment used were as described in Section 8.2.

Agarose gel electrophoresis

The reagents and equipment used were as described in Section 8.3.

Non-esterified fatty acid determinations

A commercially available kit (Nefa C, Wako Pure Chemical Industries Ltd., obtainable from Alpha Laboratories, 40 Parham Drive, East Leigh, Hampshire, England) was used, the analyses being run on a Cobas Bio centrifugal analyser.

9.3 BINDING OF THYROXINE AND TRIIODOTHYRONINE TO SERUM PROTEINS IN FELINE HYPERTHYROIDISM

Introduction

The serum binding of T4 and T3 in human thyrotoxicosis has been well-described. While the clinical aspects of feline hyperthyroidism have been documented in detail, there are no published reports of iodothyronine binding in unfractionated or fractionated sera in this disorder.

The following studies describe the investigation of iodothyronine binding in feline hyperthyroidism in unfractionated serum using the T4 uptake test, and by agarose gel electrophoresis, where possible comparing the results with post-treatment samples. The results by electrophoresis are contrasted with those obtained with normal feline serum in which T4 concentrations were artificially elevated. Studies are reported which investigate whether changes in serum non-esterified fatty acid concentrations may be responsible for T4 binding changes observed in thyrotoxicosis.

Thyroxine binding to proteins in unfractionated sera in feline hyperthyroidism

Methods

Clinical material. Sera from four cats taken before, and from 12 to 20 weeks after, successful treatment of hyperthyroidism, were used in this study. All had histories and physical findings suggestive of the disorder and palpable goitre. All had elevated total serum T₄, and three had elevated total serum T₃, concentrations. Samples from three healthy cats, three healthy humans and buffer alone were included for comparison.

Thyroxine uptake test. The T₄ uptake test was performed as described in detail in Section 8.2.

Results. Table 9.1 shows the T₄ uptake expressed as the number of counts per minute per 0.5 ml of supernatant fluid (i.e. bound to serum proteins) for each of the test samples.

There were no significant differences between the results for the healthy cats and for the untreated hyperthyroid cats, neither were there significant differences within the hyperthyroid group before and after surgery. As demonstrated in Section 8.2, the binding in human serum was consistently higher than in any of the cat samples.

Discussion. These data show that there are no differences in the gross protein binding of ¹²⁵I-T₄ between euthyroid, hyperthyroid and successfully treated hyperthyroid cats. The T₄ (and by interpolation, the T₃) uptake test cannot, therefore, be used as a diagnostic test for feline hyperthyroidism. This is probably due to the much lower affinity of feline TBG for T₄ compared with its human counterpart. It could, however, reflect a

balanced increase in the TBG and T4 concentrations in hyperthyroid feline serum, so that the number of free binding sites remained the same as in the euthyroid individual.

In order to investigate possible changes in binding to individual proteins in disease, studies were carried out using agarose gel electrophoresis.

Binding of thyroxine and triiodothyronine to serum proteins in feline hyperthyroidism. Agarose gel electrophoretic studies

Methods

Clinical material. Investigations were carried out on sera from 11 cats which satisfied the diagnostic criteria as stated in the previous section. All were domestic short-haired individuals. Five cats were female and six were male; all had been neutered. Additional case data are presented in Table 9.4. In four cases, before and after treatment sera were available for comparison. All sera had been stored at -40°C prior to analysis.

Total serum protein determinations. The total serum protein concentrations were determined by the biuret method as described in Section 4.4.

Thyroxine binding to serum proteins. The distribution of serum T4 binding was determined by agarose gel electrophoresis in tris-maleate buffer, as described in Section 8.3. Serum protein constituents were determined by densitometrically scanning gels stained with Paragon blue. Binding of ^{125}I -T4 was assessed by autoradiography and by gamma counting of protein fractions cut from the gel plates.

Results

Serum protein concentrations

Comparison between euthyroid and hyperthyroid cats.

Serum protein concentrations from the 11 cats are presented in Table 9.2. Statistical comparison with the euthyroid range is shown in Table 9.3.

Although the albumin concentrations of all pre-treatment hyperthyroid cats studied fell within the normal range, the mean was 14.7 per cent lower than that of the euthyroid group. Beta-globulin concentrations were a mean of 29.3 per cent higher in the hyperthyroid than the euthyroid animals. No other significant differences were demonstrated.

Comparison between hyperthyroid cats before and after successful surgical treatment.

Pre and post-treatment concentrations of serum albumin and globulin were compared by means of Student's paired t test. There was no significant difference for either protein fraction (albumin: $t = -0.54$, 5 d.f., $P = 0.61$; beta-globulin: $t = 0.65$, 5 d.f., $P = 0.54$).

Serum thyroxine binding. The distribution of ^{125}I -T₄ binding in hyperthyroid cat sera is shown qualitatively in Fig. 9.1 and quantitatively in Table 9.4.

Comparison between euthyroid and hyperthyroid sera.

Autoradiographically, ^{125}I -T₄ distribution was similar in euthyroid and hyperthyroid sera. In some autoradiographs, binding in the alpha-globulin zone was assessed visually as higher, and in the albumin zone as lower, than in healthy animals.

Table 9.5 presents the median, range and 25th to 75th percentiles for the per cent T₄ binding in euthyroid and hyperthyroid animals,

and shows the reduced albumin and increased alpha-1/alpha-2-globulin binding in the latter group.

The Mann-Whitney U-test was used to investigate these differences statistically. Data are presented in Table 9.6. T4 binding to albumin, to alpha-1/alpha-2-globulins taken together and to alpha-1-globulin alone was significantly different between the two groups. There was no significant difference in binding in the alpha-2-globulin zone between the two groups.

T4 binding: Differences between hyperthyroid cats before and after successful surgical treatment. Table 9.5 presents the median, range and 25th to 75th percentiles for the per cent T4 binding in hyperthyroid animals before and after successful surgical treatment, and shows apparently reduced albumin and increased alpha-1/alpha-2-globulin binding in the former group. However, when the Mann-Whitney U-test was used to investigate these data (Table 9.7), no significant differences were found.

Serum triiodothyronine binding. The quantitative distribution of ^{125}I -T3 binding in hyperthyroid cat sera is shown in Table 9.8.

Differences between euthyroid and hyperthyroid animals. Table 9.9 presents the median, range and 25th to 75th percentiles for the per cent T3 binding in euthyroid and hyperthyroid animals, and shows an apparent decrease in albumin, alpha-1-globulin, and beta-globulin binding and an increase in alpha-2-globulin binding.

The Mann-Whitney U-test was used to investigate these apparent differences statistically. Data are presented in Table 9.10. Only T3 binding to albumin and alpha-1-globulin was significantly different between the two groups. A comparison of T3 binding to

beta-globulin between the groups just failed to show significant differences.

T3 binding: Differences between hyperthyroid animals before and after successful surgical treatment. Table 9.9 presents the median, range and 25th to 75th percentiles for the per cent T3 binding in hyperthyroid animals before and after treatment and shows that, in all protein fractions except gamma-globulin, the median values for the post-treatment cases are between those for the euthyroid animals and the pre-treatment thyrotoxic individuals. In addition, the ranges of per cent T3 binding, in all protein fractions except beta-globulin for the post-treatment hyperthyroid cases, are between those of the euthyroid and pre-treatment thyrotoxic individuals. However, when the Mann-Whitney U-test was used to investigate these data (Table 9.11) the differences were not found to be significant.

Discussion

While there are a number of reports of iodothyronine binding in thyrotoxicosis in man, using either direct or reverse flow electrophoresis, no such studies have been carried out in cats. The techniques are relatively simple and require no major alterations when applied to feline sera, provided the mass of radiolabel used is not sufficient to disturb physiological equilibrium.

There is some disagreement about the binding capacity of TBG in human thyrotoxicosis. Robbins and Rall (1957, 1960) state that there is no change in capacity, whereas Werner (1978b) reported that it may be reduced. Both of these observations may be correct because Inada and Sterling (1967), using reverse flow, paper electrophoresis with glycine buffer at pH 8.6 in 23 hyperthyroid

subjects, found that 17 showed reduced and six normal TBG capacity for T4. Richards, Dowling and Ingbar (1959) reported that the capacity of TBPA is usually decreased in untreated hyperthyroid subjects but described a similar defect in subjects with other diseases. Inada and Sterling (1967) confirmed that TPBA capacity fell in all 23 hyperthyroid patients they studied. It is interesting that reduction of the capacity of these proteins in thyrotoxicosis is in a direction which would tend to exaggerate the disorder.

The present study clearly demonstrates a reduction in the proportion of T4 bound by albumin and an increase in the proportion bound by alpha-globulin in hyperthyroid cats. There is, of course, a reciprocal relationship between the two and a fall in one must be accompanied by a rise in the other. It is possible, therefore, that the change may be in either the albumin or the alpha-globulin binding.

In human thyrotoxicosis, the serum albumin concentration was found to fall by 15 per cent (Lewis and McCullagh, 1944), but the reason for this change was unexplained. It is of considerable interest that, in hyperthyroid cats, the mean albumin concentration was also 15 per cent lower than for euthyroid animals. This may either result from a reduced rate of formation or an increased rate of degradation. It is possible that the hepatic damage which is a feature of feline and human hyperthyroidism may result in reduced albumin synthesis. An elevated rate of catabolism such as occurs in hyperthyroidism could result in increased albumin breakdown.

Robbins and Rall (1957) felt that a 15 per cent fall in human albumin concentration would not affect its binding capacity

significantly. However, it is interesting to speculate that in cats, where the affinity of serum proteins for T4 is much lower than in man, a reduction in albumin concentration may alter the binding equilibrium so that alpha-globulin plays a relatively larger role in T4 carriage.

It is possible that a factor present in the serum of hyperthyroid cats may inhibit T4 binding to albumin but these studies provide no information on this hypothesis.

An alternative explanation for the relative increase in alpha-globulin T4 binding in feline hyperthyroidism is an increase in this protein's capacity. This could result from either an increase in its affinity or in its serum concentration. Because a binding protein's affinity is generally genetically determined, any change in alpha-globulin capacity must result from a change in its serum concentration. Unfortunately, measurement of the concentration of the specific globulin of cats which binds T4 was not possible as its physicochemical and immunological characteristics remain unknown. It would appear from the T4 uptake test, however, that the overall binding of T4 in hyperthyroidism remains unchanged, and that the change is simply restricted to a shift from albumin to alpha-globulin binding.

In human thyrotoxicosis, the TBG capacity does not change after successful treatment (Robbins and Rall, 1957). In this study, the protein binding of ^{125}I -T4 by both albumin and alpha-globulin in hyperthyroid animals tended to revert towards the normal ranges with successful treatment, although the results were not quite statistically significant.

With regard to T3, only binding to albumin and alpha-1-globulin was significantly different in hyperthyroid and euthyroid cats. The effect of hyperthyroidism on albumin concentrations has been referred to previously. However, only small percentages of T3 are bound by these two proteins in the cat and these changes are unlikely to be important in vivo.

The changes in T3 binding by beta-globulin in hyperthyroid cats just failed to show statistical significance. It is interesting, however, that the beta-globulin concentration of hyperthyroid cats was significantly higher than the normal range. Speaking of domestic animals in general, Kaneko (1980b) stated that increases in beta-globulins alone are infrequent and are found only in association with active liver disease, suppurative dermatopathies and occasionally the nephrotic syndrome. The hyperthyroid individuals in this series had neither infected skin lesions nor the nephrotic syndrome but did show alterations in indices of liver function (see Chapter 10). The hepatopathy of feline hyperthyroidism has been referred to previously. It is interesting to speculate that this resulted in the production of a beta-globulin with abnormal T3 binding capacity, causing the lowered T3 binding in this fraction. While the differences were not statistically significant, the changes in T3 binding tended to revert towards the euthyroid range with successful surgical treatment.

Three cats (FT/HE/11/83, FT/HE/26/83 and FT/HE/27/83) showed different serum T3 binding patterns from the remainder, with much higher binding in the beta and gamma-globulin fractions with a consequent reduction in the alpha-2-globulin. One of these (FT/HE/26/83) was the only hyperthyroid cat to show higher T4

binding in the albumin than in the alpha-globulin fraction. It is possible that a larger study may reveal other patterns of change in iodothyronine binding than those described in detail here and further work in this area is required.

Thyroxine binding in normal feline serum. Effects of increments of added thyroxine

Introduction

The previous section demonstrated that there is increased alpha-1/alpha-2-globulin binding and decreased albumin binding in thyrotoxic cats and suggested possible reasons for this abnormality. This study was carried out to confirm that this change was not simply a result of increased T4 concentrations per se but reflected alterations in T4 binding proteins.

Method. Known amounts of T4 were added to aliquots of a sample of normal feline serum to give concentrations in the range of 95 nmol/l to 440 nmol/l. The T4 was added as a 20 μ l volume to 0.48 ml aliquots of serum, the T4 solutions (concentrations approximately 2.5 μ mol/l, 5.0 μ mol/l, 7.5 μ mol/l, 10.0 μ mol/l, 12.5 μ mol/l and 15 μ mol/l) being prepared in diluent. The resultant solutions were assayed for T4 as described in Section 6.1 to determine the actual concentrations.

Serum thyroxine binding for each concentration was determined as described in Section 8.3 using agarose gel electrophoresis with tris-maleate buffer. The results were assessed qualitatively by autoradiography and quantitatively by cutting and gamma counting the electrophoresed gels.

Results. Qualitative results of autoradiographic studies are presented in Fig. 9.2 and show distribution of the radiolabel in the albumin and alpha-1/alpha-2-globulin zones.

Quantitative results are presented in Table 9.12.

Increasing the T₄ concentration more than ten fold above the basal concentration of 29 nmol/l failed to reproduce the distribution of binding seen in hyperthyroid cats. There was an increase in the proportion of T₄ bound to albumin at 440 nmol/l and a decrease in the proportion bound to alpha-1/alpha-2-globulin, changes which were the converse of those seen in naturally-occurring hyperthyroidism. No changes in the percentages of labelled T₄ found in other globulin fractions were detected.

Discussion

This study was carried out to determine whether the increased alpha-1/alpha-2-globulin binding and reduced albumin binding in feline hyperthyroidism might be a function of hyperthyroxinaemia per se. The results clearly exclude this possibility, the changes seen at the highest concentration of T₄ examined being, in fact, the converse of those seen in naturally-occurring hyperthyroidism. At lower T₄ concentrations, no significant changes in distribution were found.

In human serum, the addition of increasing quantities of T₄ results in a decreasing proportion of labelled T₄ bound by TBG and an increasing proportion bound by albumin (Woeber, 1971). The proportion bound by TBPA initially increases and then declines.

Tanabe, Ishii and Tamaki (1969) investigated the effects of added T₄ on the protein association of radiothyroxine after cellulose acetate electrophoresis of serum from healthy cats. They found that the radioactivity in the area immediately cathodal to albumin did not disappear or decrease when T₄ was added and suggested that this band was therefore post-albumin and not TBG.

However, these findings were probably due to very poor separation of albumin and alpha-1/alpha-2-globulin in their studies.

The results reported here support the hypothesis that the changes in distribution of labelled T4 in hyperthyroidism are due to changes in the binding capacity of albumin or the alpha-1/alpha-2-globulins, and are not simply a feature of raised T4 concentrations. Parenthetically, these observations provide additional evidence that T4 migrating in the alpha-1/alpha-2-globulin zone is not free T4. If this were the case, there would have been an increasing fraction in the alpha-1/alpha-2 zone and a decreasing fraction in the albumin zone with increasing exogenous T4, reflecting the progressive saturation of binding proteins and increasing free T4 concentration.

Changes in binding capacity may be due to inhibition of T4 binding. One such group of inhibitors is non-esterified fatty acids (NEFA) (Tabachnick, 1964) and experiments were performed to investigate this possibility.

Are increases in serum non-esterified fatty acids responsible for changes in thyroxine protein binding in feline hyperthyroidism?

Introduction. Non-esterified fatty acids are capable of displacing T4 from binding sites on human albumin. In man, the concentration of NEFA in serum is significantly increased in thyrotoxicosis. Investigations were therefore carried out to determine whether there might be a similar increase in NEFA in feline hyperthyroidism which might result in displacement of binding from albumin and increased binding to alpha-1/alpha-2-globulin.

Method

Clinical material. Sera from three hyperthyroid cats were used in this study. All had histories and physical findings

suggestive of the disorder and palpable goitre. All had elevated total serum T4 and T3 concentrations and elevated percentage alpha-1/alpha-2-globulin T4 binding. In one case, a sample taken 13 weeks after successful surgical treatment was also tested. Samples from nine healthy cats were included for comparison. All animals had been fasted for 12 hours and were conscious when bled.

Non-esterified fatty acid determinations. Serum NEFA were measured by courtesy of Dr. R. A. Riemersma using a commercially available enzymatic kit method. The technique relies on the acylation of coenzyme A (CoA) by fatty acids in the presence of added acyl CoA synthetase. The acyl CoA thus produced is oxidised by added acyl CoA oxidase with generation of hydrogen peroxide. In the presence of peroxidase, this permits the oxidative condensation of 3-methyl-N-enthyl-N-(beta-hydroxyethyl)-aniline with 4-aminoantipyrine to form a purple coloured adduct which is measured colorimetrically at 550 nm.

The coefficient of variation of the method was seven per cent.

Results. Clinical data on the animals studied and the results of biochemical investigations are presented in Table 9.13.

There is no obvious difference between the NEFA concentrations of euthyroid and hyperthyroid animals. In the single animal in which samples were checked before and after surgery, there was a fall in the NEFA concentration with successful treatment but it is not possible to draw a firm conclusion from this single observation.

Discussion. Many methods of NEFA determination require their prior extraction from serum. These approaches are time-consuming, potentially hazardous and not easily automated. In this study, an enzymatic method was used which was accurate, precise and rapid.

Blood for analysis must be collected after an overnight fast because the concentration of circulating NEFA is increased by the ingestion of food. Blood collected in heparinised containers is not suitable for the test, as heparin is known to stimulate the activity of lipoprotein lipase. Some anaesthetics also interfere with the method and blood should be collected from conscious patients.

The effect of various anionic compounds in inhibiting the binding of T4 by human serum albumin is well-known. Thus, according to decreasing effectiveness, the following compounds are capable of displacing T4 from its binding sites to albumin: oleate, linoleate, dodecyl sulphate, palmitate, laurate, octanoate and salicylate (Tabachnick, 1964). For example, oleate at a molar ratio to albumin of 3.1:1 causes a reduction of approximately 70 per cent in the ability of the protein to bind T4. Conversely, defatted albumin has an increased affinity for T4 (Tabachnick, 1964).

The ability of various anions to displace T4 from binding sites on albumin may be of physiological significance. In human hyperthyroidism, the concentrations of NEFA in blood rise from one to two moles fatty acid per mole of albumin (Rich, Bierman and Schwartz, 1959). This may lead to increased removal of T4 from binding sites on plasma albumin and be one of the factors involved in producing the acceleration in the rate of disappearance of T4 from the circulation that occurs in human thyrotoxicosis (Tabachnick, 1964; Koppers and Palumbo, 1972).

In this study, NEFA were measured in thyrotoxic feline sera to see whether this might explain the decreased albumin binding. There were no obvious differences in the results between the hyperthyroid and euthyroid animals. However, NEFA were only measured in three

hyperthyroid sera and this hypothesis warrants further investigation.

General conclusions on iodothyronine binding in feline hyperthyroidism

These studies allow the following general conclusions to be made about iodothyronine binding in feline hyperthyroidism:

(i) There is no change from normal in the percentage of T4 bound in whole serum, as judged from the results of the T4 uptake test. This test is therefore of no value in the diagnosis of feline hyperthyroidism.

(ii) The percentage T4 bound to albumin is decreased and the percentage T4 bound to alpha-globulin is increased. However, the percentage binding to one protein is inversely proportional to the other. Thus, these changes may reflect the decrease in serum albumin concentrations which occur in hyperthyroidism, inhibition of T4 binding to albumin or an increased TBG concentration. They are not the result of hyperthyroxinaemia per se, nor, in the small number of cases studied, were they due to blockage of binding of T4 to albumin by NEFA.

(iii) The percentage of T3 bound to albumin and alpha-1-globulin is significantly decreased but these changes are unlikely to be important in vivo. Although not quite statistically significant, the percentage binding of T3 to beta-globulin is reduced, which may reflect an increased synthesis of beta-globulin with abnormal binding properties.

(iv) After successful surgical treatment of feline thyrotoxicosis, T4 and T3 binding tends to revert to that seen in euthyroid animals, although these changes do not reach statistical significance.

9.4 BINDING OF THYROXINE AND TRIIODOTHYRONINE TO SERUM PROTEINS

IN EXPERIMENTALLY-INDUCED FELINE HYPOTHYROIDISM

Introduction

There have been a number of reports describing the serum protein binding of T₄ and T₃ in human hypothyroidism. Such studies have not been carried out in cats because no cases of confirmed, naturally-occurring, acquired, feline hypothyroidism have been documented.

This section reports on the serum protein binding of T₄ in unfractionated serum, and T₄ and T₃ after agarose gel electrophoresis, in two cats in which hypothyroidism was produced experimentally.

Methods

Clinical material. Sera from two five-year-old, adult female, domestic short-haired cats, taken before and 30 weeks after successful thyroid ablation with ¹³¹I, were used in this study. Hypothyroidism was confirmed by the absence of T₄ and T₃ in post-treatment serum samples and subsequently by the failure to produce a serum T₄ or T₃ response by TSH stimulation. Details of these experimental procedures are presented in Chapters 11 and 13. All sera were stored at -40°C prior to analysis.

Thyroxine uptake test. The T₄ uptake test was performed, as described in Section 8.2, on paired sera from each cat before and after thyroid ablation. Samples from three healthy cats, three healthy humans and buffer alone were included for comparison.

Results. Table 9.14 shows the number of counts per minute per 0.5 ml of supernatant fluid (i.e. bound to serum proteins) for each of the test samples. There were no obvious differences between the results for the normal cats and the experimental cats before

treatment or between the experimental cats before or after thyroid ablation. As shown in Section 8.2, the T4 binding in human serum was consistently higher than in any of the cat samples.

Discussion. The data presented clearly show that the T4 (and by interpolation, the T3) uptake test cannot be used for the diagnosis of feline hypothyroidism. An essential requirement for the use of the test is a high-affinity, serum iodothyronine binding protein, and the lack of change after induction of hypothyroidism in these cases probably reflects the much lower affinity that feline TBG has for T4 than its human counterpart. It could, however, reflect a balanced decrease in the TBG and T4 concentrations in hypothyroid cat sera, so that the number of free binding sites remained the same as in the euthyroid individual.

In order to investigate possible changes in binding to individual proteins, studies were carried out using agarose gel electrophoresis.

Agarose gel electrophoretic studies

Methods

Clinical material. The clinical material was as described in the previous study.

Total serum protein determinations. The total serum protein concentrations were determined by the biuret method as described in Section 4.4.

Thyroxine binding to serum proteins. The distribution of serum T4 binding was determined by agarose gel electrophoresis in tris-maleate buffer, as described in Section 8.3. Serum protein constituents were determined by densitometrically scanning gels stained with Paragon blue. Binding of ^{125}I -T4 was assessed by

autoradiography and by gamma counting of protein fractions cut from the gel plates.

Triiodothyronine binding to serum proteins. This study was performed as above and as described in detail in Section 8.3. The distribution of the radiolabel was determined by cutting and gamma counting the electrophoresed gels according to their protein fractions.

Results

Serum protein concentrations. Data on serum protein concentrations of the samples used in the studies are presented in Table 9.15.

The gamma-globulin concentration rose after thyroid ablation such that in cat 1, it was above the upper limit of the reference range.

Thyroxine binding to serum proteins. Fig. 9.3 shows autoradiographs of sera from the two animals before and after thyroid ablation and illustrates that there are no qualitative differences due to induced hypothyroidism.

Table 9.16 provides quantitative data on the ^{125}I -T₄ binding in the same samples. There are no obvious differences before and after ablation and values for each of the fractions binding T₄ lie within one sd of the mean of the euthyroid group.

Triiodothyronine binding to serum proteins. Table 9.17 presents data on ^{125}I -T₃ binding in serum samples taken before and after thyroid ablation.

There are no obvious differences between the pre and post-ablation samples and all values lie within two sds of the mean of the euthyroid range.

Discussion

There is some disagreement about the capacity of thyroid hormone binding proteins in human hypothyroidism. In an early study, Albright, Larson and Deiss (1955) found that there was no change in the T4 capacity of what they termed "thyroxine binding proteins" (TBP, now called TBG) in hypothyroidism, although the proteins were relatively unsaturated with respect to T4. However, Robbins and Rall (1957, 1960) found that the TBP concentration was slightly but significantly elevated in all seven hypothyroid patients studied. Bellabarba, Inada, Varsano-Aharon and Sterling (1968) and Oppenheimer (1968) found that the TBG concentration was increased by ten to 30 per cent in hypothyroid subjects. Inada and Sterling (1967) showed that both situations may apply. Using reverse flow paper electrophoresis with glycine acetate buffer at pH 8.6, five of 16 hypothyroid subjects had normal, and 11 of 16 increased, TBG capacity for T4. The TBPA capacity was normal in all subjects studied. An increase in TBG capacity is in a direction which would tend to exaggerate the change in free hormone concentrations in the disorder.

Robbins and Rall (1957) showed that successful treatment of hypothyroidism in two patients produced a fall in TBP to normal concentrations. This occurred with T3 therapy (which could not have increased the serum T4 concentrations) as well as with T4 therapy. No attempt was made to reverse the hypothyroid state in the cats in this study.

Both cats showed an increase in their serum gamma-globulin concentrations after thyroid ablation. Such changes have also been demonstrated in Hashimoto's disease in man (Roitt, Doniach, Campbell

and Vaughan Hudson, 1956) and experimental thyroidectomy in the rat (Levin and Leatham, 1942) and will be discussed further in Chapter 11.

As no cases of naturally-occurring, acquired, feline hypothyroidism have been definitively diagnosed, this study of T4 and T3 binding in two cases of the experimentally-induced condition is the first to be published. No significant binding changes were documented. However, it is possible that investigations on a much larger number of cases would reveal subtle changes not demonstrated here.

9.5 BINDING OF THYROXINE AND TRIIODOTHYRONINE TO SERUM PROTEINS IN HEALTHY EUTHYROID CATS WITH HYPERTHYROXINAEMIA AND/OR HYPERTRIIODOTHYRONINAEMIA

Introduction

Euthyroid hyperthyroxinaemia and hypertriiodothyroninaemia have been well-documented in man. The conditions have not been previously reported in cats.

In Chapter 7, details were given of two apparently healthy, euthyroid cats, one of which had grossly elevated T4 and T3 and one normal T4 but grossly elevated T3 concentrations. This section describes investigations into the serum protein binding of iodothyronines in these animals.

Methods

Clinical material. Detailed information on these two animals was presented in Chapter 7 and is summarised in Table 7.9. Sera were stored at -40°C until tested. In each case, the abnormal iodothyronine concentrations were confirmed by repeating the RIAs.

Total serum protein determinations. The total serum protein concentrations were determined by the biuret method as described in Section 4.4.

Thyroxine binding to serum proteins. The distribution of T₄ binding was determined by agarose gel electrophoresis in tris-maleate buffer at pH 7.4 as described in Section 8.3. Serum protein constituents were determined by densitometrically scanning gels stained with Paragon blue. Binding of ¹²⁵I-T₄ was assessed by autoradiography and by gamma counting of protein fractions cut from the gel plates.

Triiodothyronine binding to serum proteins. This study was performed as above and as described in detail in Section 8.3. The distribution of the radiolabel was determined by cutting and gamma counting the labelled, electrophoresed gels.

Results

Serum protein concentrations. The serum protein concentrations of the two cats are presented in Table 9.18.

The total serum protein concentrations of both cats were elevated. With cat FT/NC/9/82, this was due, in the main, to a gross increase in the gamma-globulin concentration, although the beta-globulin concentration was also elevated. With cat FT/NC/3/82, only the beta-globulin fraction was elevated.

Thyroxine binding to serum proteins. Fig. 9.4 shows the autoradiographs obtained with the two test sera and compares them to a normal autoradiograph and electrophoretogram. Table 9.19 provides quantitative data on T₄ binding in the two sera.

(i) FT/NC/9/82. Both qualitatively and quantitatively, the serum from this cat showed relatively little albumin binding of

^{125}I -T₄. Almost all of the T₄ was carried in the alpha-1 and alpha-2-globulin zones, although the beta and gamma-globulin fractions also carried elevated amounts of radiolabel.

(ii) FT/NC/3/82. Both qualitatively and quantitatively, the serum from this cat showed reduced albumin binding of ^{125}I -T₄. Qualitatively, no other abnormalities were demonstrated but quantitatively there was elevated binding in the alpha-1-globulin with normal percentage binding in the alpha-2-globulin zones. Minimal amounts of radiolabel were found in the other protein zones.

Triiodothyronine binding to serum proteins. Table 9.20 presents data on T₃ binding in the two sera.

(i) FT/NC/9/82. T₃ carriage in the alpha-1 and alpha-2-globulin zones was marginally and markedly reduced respectively. This was accompanied by a gross elevation in T₃ carriage in the gamma-globulin fraction.

(ii) FT/NC/3/82. T₃ carriage in the alpha-1-globulin fraction was marginally reduced but this was probably not significant. No other abnormalities were noted.

Discussion

Euthyroid hyperthyroxinaemia, with or without hypertriiodothyroninaemia has been well-documented in man (reviewed by Borst, Eil and Burman, 1983). The numerous causes have been reviewed in Section 7.4. The condition has not been recorded previously in the cat. Euthyroid hypertriiodothyroninaemia, with normal T₄ concentrations, has not been recorded in either man or the cat. The causes of the states in these two animals are difficult to explain with the relatively small amount of data available.

FT/NC/9/82. Attempts to explain the binding changes seen in this cat may be considered under three headings:

(i) Interference with methodologies. The elevated gamma-globulin concentration in this animal, together with the increased T3 binding in this region, suggest that thyroid hormone binding immunoglobulins may be in some way responsible for the binding abnormalities seen. Such immunoglobulins have been reported in the serum of human patients with autoimmune thyroid disease (Premachandra and Blumenthal, 1967; Staeheli, Vallotton and Burger, 1975; Jorgensen, Skovsted and Jensen, 1979). They cause interference in the direct RIA of T4 and T3 and thus produce incorrect values. The type of interference produced depends on the separation method involved in the assay (Beckett, Todd, Hughes and Campbell, 1983). The double antibody system, as employed in these studies, does not precipitate human gamma-globulin and thus the tracer fraction bound to it is not measured. This produces spuriously high thyroid hormone concentrations (Wu and Green, 1976; Ginsberg, Segal, Erlich and Walfish, 1978).

In the case under discussion, no T4 binding was demonstrated in the gamma-globulin region after serum electrophoresis. The presence of antibodies against T3 (anti-T3) alone, as has been described in man (Wu and Green, 1976), would neither explain the elevated T4 concentration nor its increased binding in the alpha-1/alpha-2-globulin region, unless antibodies to T4 (anti-T4), which migrated for some unexplained reasons in this protein fraction on electrophoresis, were also present.

(ii) Changes in serum protein concentrations. In human subjects, changes in the concentrations of various serum proteins may produce profound alterations in thyroid hormone binding patterns.

An inherited increase in TBG concentration was the first recognised cause for alterations in human serum iodothyronine binding (Beierwaltes and Robbins, 1959). Because the protein binds both T4 and T3, affected people have elevated TBG binding and elevated serum concentrations of both iodothyronines (Borst, Eil and Burman, 1983). In the cat under discussion, the serum T4 and T3 were both elevated and the alpha-globulin binding of T4 was markedly increased. However, T3 binding in the alpha-globulin fraction was actually decreased, with a marked increase in the gamma-globulin zone.

A further inherited thyroid hormone binding protein abnormality which has been described in man is increased TBPA concentrations. TBPA was not demonstrated in any cat in the studies in Chapter 8, but it is not known whether this was a feature of the separation systems used or whether the protein does not occur in the cat. As observed previously, Tata (1959) suggested that in certain buffer systems, human TBPA migrated electrophoretically as an inter-alpha-globulin. If this were the case in cat serum, with the system used, increased TBPA would explain the T4 binding changes noted in this patient. However, because human TBPA does not bind T3, individuals with excess TBPA have normal T3 concentrations (Moses, Lawlor, Haddow and Jackson, 1982) whereas they were elevated in the cat under discussion.

Analbuminaemia is a rare serum protein variant in man (Stockigt, Stevens, White and Barlow, 1983). A corresponding abnormality in the cat under discussion would explain the T4 binding changes seen but is not supported by the results of serum protein electrophoresis.

(iii) Changes in the iodothyronine binding affinity of serum proteins. The iodothyronine binding changes seen in this cat might, in part, be explained by abnormalities in the binding affinity of its serum proteins. An abnormal albumin variant which failed to bind T4 would explain the reduced albumin binding and the relative increase in alpha-1/alpha-2-globulin binding in this case. However, the reduction in the overall number of binding sites would tend to result in a lowered total T4 whereas in this case it was increased. Such an abnormal variant of albumin has not been described in man and would still fail to explain the altered T3 binding seen in this case.

Circulating inhibitors of serum T4 and T3 binding resulting in a raised FT4 would also increase the free ^{125}I -T4 and ^{125}I -T3 concentrations and produce the binding abnormalities noted. The possible influence of elevated serum NEFA concentrations on the inhibition of iodothyronine binding in this animal is discussed in Section 9.6.

An inhibitor of thyroid hormone binding to serum proteins in the serum of some human patients with non-thyroidal illness has been described (see Section 9.6). However, the cat under discussion was apparently healthy and remained so over a follow-up period of three years and it is unlikely that this could account for the changes seen.

FT/NC/3/82. The abnormalities in this case are also extremely difficult to explain with the data available. It is interesting that while the T3 concentration is grossly elevated, there are minimal T3 binding changes, while there are obvious T4 binding abnormalities despite a normal T4 concentration.

(i) Interference with methodologies. The elevated T3 concentration without a concomitant increase in T4 could be explained by anti-T3 interference in the separation stage of the assay in the absence of anti-T4. However, the percentage of T3 found in the gamma-globulin zone on electrophoresis was within the normal range.

(ii) Changes in serum protein concentrations. As discussed earlier in this chapter, the iodothyronine serum protein binding changes seen in hyperthyroidism may reflect changes in serum protein concentrations. In the cat under discussion, the decreased albumin and increased alpha-globulin binding of T4, the decreased T3 binding by alpha-1-globulin and the elevated beta-globulin concentration are all suggestive of hyperthyroidism due to T3 toxicosis. For the reasons discussed in Section 7.4, this is an unlikely explanation of the changes noted.

(iii) Changes in the iodothyronine binding affinity of serum proteins. As described in detail in Section 7.3, this cat had been involved in a road traffic accident three weeks before sampling but, apart from thiopentone sodium anaesthesia for mandibular symphysis repair immediately after the accident, no other drugs had been given and the cat had been clinically normal for two weeks. As will be discussed in detail in the following section, inhibitors of thyroid hormone binding to serum proteins may be present in nonthyroidal illnesses and may also result from soft tissue injury. However, it is difficult to explain how the earlier road traffic accident and associated anaesthesia might have produced apparent hypertriiodothyroninaemia.

The specific reasons for the binding changes seen in these cats cannot be determined from the data available. Unfortunately, neither owner would allow further blood samples to be taken from his animal. Further information on serum T4 and T3 binding by the T4 or T3 uptake tests, quantitative determination of TBG concentrations and the measurement of FT4 and FT3 concentrations would have added much to the understanding of these cases but largely await the development and validation of suitable methodologies for use with cat serum.

9.6 BINDING OF THYROXINE AND TRIIODOTHYRONINE TO SERUM PROTEINS IN SELECTED CATS WITH EUTHYROID PATHOLOGICAL STATES

Introduction

The thyroxine binding proteins of human serum have been examined in a variety of pathological situations. Apart from their general interest, such studies have been used to evaluate the physiological importance of the thyroxine-protein interactions. No corresponding investigations have been carried out in cats.

During the studies described in Chapter 7, two cats with definitively diagnosed disease were found to have grossly subnormal T4 concentrations. One also had hypertriiodothyroninaemia. This section describes investigations into the serum protein binding of iodothyronines in these animals.

Methods

Clinical material. The case details of the two cats are presented in Table 9.21. In each case, the abnormal serum iodothyronine concentrations were confirmed by repeating the RIAs.

Case number 124550 was sampled on two occasions (124550a and 124550b respectively) six months apart. On the second occasion, the presenting complaints were weight loss and decreased activity. Physical examination confirmed slight weight loss and flea infestation but no further abnormalities were noted. The cat died suddenly eight weeks later but post-mortem examination was not permitted.

All sera were stored at -40°C prior to analysis.

Total serum protein determinations. The total serum protein concentrations were determined by the biuret method as described in Section 4.4. Sample 124550b was not tested.

Thyroxine binding to serum proteins. The distribution of serum T4 binding was determined by agarose gel electrophoresis in tris-maleate buffer as described in Section 8.3. Serum protein constituents were determined by densitometrically scanning gels stained with Paragon blue. Binding of ^{125}I -T4 was assessed by autoradiography and by gamma counting of protein fractions cut from the gel plates. Sample 124550b was not tested.

Triiodothyronine binding to serum proteins. This study was performed as above and as described in detail in Section 8.3. The distribution of the radiolabel was determined by cutting and gamma counting the electrophoresed gels. Sample 124550b was not tested.

Results

Serum protein concentrations. These data are presented in Table 9.22.

All values for sample 124550a were within the normal reference ranges. The serum from cat FT/NC/5/82 was markedly lipaemic resulting in an incorrectly elevated total protein

concentration. The serum protein concentration reverted to normal after delipidation by ether extraction.

Thyroxine binding to serum proteins. Fig. 9.5 shows autoradiographs obtained with the two test sera and compares them to a normal autoradiograph and electrophoretogram. Table 9.23 provides quantitative data on T4 binding in the two sera.

(i) FT/NC/5/82. Both qualitatively and quantitatively, this serum showed minimal albumin binding of ^{125}I -T4. Almost all of the radioactivity was carried in the alpha-1 and alpha-2-globulin zones. However, within these zones, the distribution was changed from that seen in healthy cats. From the autoradiograph, it could be seen that the binding of the radiolabel had been shifted cathodally, with little ^{125}I -T4 being present in the leading half of the alpha-1-globulin. The total radiolabel in this zone remained within the normal range but there was a significant increase in the percentage present in alpha-2-globulin. A slightly higher percentage of ^{125}I -T4 was also found in the beta-globulin, probably reflecting this cathodal shift of binding.

(ii) 124550a. This serum showed normal albumin binding of T4. Autoradiographically, binding was diffuse throughout the alpha-globulin zone. This resulted in the percentage binding in the alpha-1-globulin being toward the low end of the normal range, with a corresponding increase in the alpha-2-globulin.

Triiodothyronine binding to serum proteins. Table 9.24 presents these data.

(i) FT/NC/5/82. This serum showed reduced alpha-2-globulin and increased gamma-globulin binding of ^{125}I -T3. Binding in the other protein fractions was within the appropriate reference ranges.

(ii) 124550a. No abnormalities of T3 binding were noted in this cat.

Discussion

FT/NC/5/82. This cat sustained a fractured pelvis in a road traffic accident five days before sampling. It was treated conservatively and required no medication. Serum samples were grossly lipaemic. In man, lipaemia may occur in starvation (Rogers, 1977). As this cat had not eaten since the accident, the lipaemia probably reflected fat mobilisation in an anorectic animal. Both thyroid hormone RIA and protein binding studies were carried out on whole serum that had not been ether extracted.

(i) Thyroid hormone concentrations. Drug therapy may reduce total T4 concentrations (reviewed in Section 3.6) but as no drugs had been administered, may be discounted as a cause of the low T4 concentration in this animal.

The lipaemia noted reflected elevated NEFA concentrations. However, it is unlikely that this resulted in the low T4 concentration because, in human serum, total T4 RIA is not influenced by NEFA concentrations (Rootwelt, 1975).

It is possible that the low serum T4 concentration resulted from hypothyroidism. In man, a normal serum T3 concentration may be preserved at the expense of T4 in the early stages of the disease (Seth, Toft and Irvine, 1976). However, no cases of naturally-occurring, acquired hypothyroidism in cats have been described and this animal was healthy prior to the road traffic accident. Hypothyroidism is, therefore, an unlikely diagnosis in this case.

The low T4 concentration of this animal probably resulted from a condition analagous to the low T4 state of medical illness of man. This is a specific category of the euthyroid sick syndrome

(reviewed in Section 3.6). In part, it may result from the inhibition of binding of T4 to serum proteins by either an IgM antibody, an immune complex with IgM, or a substance that shares with IgM several physicochemical and antigenic characteristics (Chopra et al., 1979), as well as from increased T4 disposal.

The T3 concentration of the cat under discussion was within the normal range. In man, the low T4 state of medical illness is usually accompanied by a low total T3 concentration. A group of dogs with a variety of medical illnesses in an intensive care unit had subnormal total T4 concentrations (Ferguson, 1984), but their T3 concentrations were not reported. No such studies have been carried out in cats.

(ii) Changes in the iodothyronine binding affinity of serum proteins. It is possible that the reduced albumin binding of T4 in this case was induced by elevated NEFA concentrations in the lipaemic serum, as may be seen in man (Section 9.3). The subsequent addition of ^{125}I -T4 would result in an increased free concentration of the radiolabel which would migrate in the position shown in Table 9.5.

The T4 and T3 binding changes seen in cat FT/NC/9/82, (discussed in the previous section), were very similar to those seen in this case and it is interesting to speculate that there may have been a common cause. Both animals showed elevated total protein concentrations. The serum from FT/NC/9/82 was slightly lipaemic, although this was not considered to be sufficient to require ether extraction. In retrospect, it is possible that this gave an artificially high, total protein concentration, which was reflected in elevations of the individual protein fractions. A concurrently

elevated serum NEFA might have resulted in the binding changes observed. A cause for the slight lipaemia in this case is not obvious from the history.

Considerable soft tissue damage resulted from the fractured pelvis sustained by cat FT/NC/5/82. Chopra, Solomon, Chua Teco and Eisenberg (1982) reported that extrathyroidal tissue of man and the rat contained a potent inhibitor of the binding of thyroid hormones to serum proteins which acted by reducing the binding affinity of thyroid hormones to serum proteins and not by reducing the number of binding sites. The tissue inhibitor was similar to that described previously in the serum of some critically ill patients (Chopra et al., 1979; Oppenheimer, Schwartz, Mariash and Kaiser, 1982) and they suggested that tissue damage might result in its leakage into the circulation.

In man, serious illness is accompanied by a sharp fall in serum TBPA concentration but as TBPA has a relatively minor role in T4 transport, this is not thought to be of clinical significance (Oppenheimer, 1968). For reasons discussed in Chapter 8, the importance of this observation with respect to cats is unknown.

An abnormal albumin variant which failed to bind T4 might result in the changes seen in this case. As discussed in Section 9.5, such an abnormal variant has not been described in man and would not explain the altered T3 binding in this case.

In summary, the most likely causes of the laboratory changes seen in cat FT/NC/5/82 are the low T4 state of medical illness, together with lipaemia resulting from anorexia. It would have been interesting to repeat the tests in the recovered animal but it was lost to follow-up.

(i) Thyroid hormone concentrations. This cat initially showed low serum total T4 and elevated serum total T3 concentrations (124550a). However, a repeat blood sample six months later, shortly before the animal died, revealed normal T4 and T3 concentrations (124550b). It is unlikely that the results obtained with the first sample were methodological in origin as they were confirmed on repeat analysis.

Treatment with exogenous T3 would result in the abnormalities seen in 124550a, but such treatment can be excluded in this case.

T3 toxicosis would cause high serum T3 and low serum T4 concentrations but this state has not been described in the cat and compatible physical signs were not present when the animal was first examined.

It is interesting to speculate that the normal T3 concentration in the second sample was due to the reduction of a naturally-occurring, high T3 concentration as the result of terminal disease in this cat. An analogous situation occurs in the so-called hypermetabolic state of "T4 toxicosis" in man, in which the serum total T3 concentration is artificially decreased in hyperthyroidism because of concomitant fasting or systemic illness (Turner, Brownlie and Sadler, 1975; Joasoo, 1975; Birkhauser, Burer, Busset and Burger, 1977; Engler, Donalson, Stockigt and Taft, 1978). In this syndrome, the previously normal serum T3 concentration enters the thyrotoxic range during a patient's convalescence from serious, non-thyroidal disease. While this is a possible explanation for the discrepancy in the total T3 concentration in the two sera, it does not explain the low T4 concentrations found on each occasion.

Although the cat had skin and ear pathology when initially sampled, this was not severe enough to have resulted in a low T4 concentration, and the euthyroid sick syndrome would have caused a low, rather than an elevated, serum total T3 concentration.

As discussed in Section 9.5, T3 antibodies may cause spuriously high T3 concentrations when measured by RIA. It is possible that these may have disappeared from the serum six months later. However, this would not explain the low serum total T4 concentration in sample 124550a.

In man, familial decrease in TBG concentration results in reduced serum total T4 and T3 concentrations (Refetoff and Selenkow, 1968). However, a comparable abnormality in the cat discussed here would not explain the initial elevated T3 concentration, nor the return to the normal range in the second sample.

(ii) Changes in the iodothyronine binding affinity of serum proteins. The only abnormality noted was a diffuse binding of T4 throughout the alpha-globulin zone. Such a change could result from acquired or inherited TBG excess. However, as discussed in Section 9.5, such abnormalities in man result in elevations in both T4 and T3 concentrations, whereas T3 alone was elevated in this case.

It is possible that elevated NEFA concentrations might increase the percentage T4 bound to alpha-globulin while inhibiting its binding to albumin. However, qualitative and quantitative albumin binding of T4 was normal in this case.

It would have been of interest to determine the serum protein concentrations and the distribution of iodothyronine binding in serum taken from this animal at the second visit (124550b). Unfortunately, insufficient sample remained to carry out these tests.

In human medicine, there is now a large body of literature on thyroid function in non-thyroidal illness. This interesting field awaits investigation by the veterinary endocrinologist.

10. FELINE HYPERTHYROIDISM

10.1 INTRODUCTION

Until 1979, no cases of naturally-occurring feline hyperthyroidism had been definitively diagnosed. Currently, thyrotoxicosis is recognised as a common disease of older cats (Peterson 1984) but it is unclear whether there has been a real increase in the prevalence of the disease or whether clinical awareness and the availability of thyroid hormone RIA has led to an increase in the diagnosis of the condition.

Only three major independent series of cases of feline hyperthyroidism have been described in detail: ten cases by Holzworth et al. (1980), 24 cases by Hoenig et al. (1982) and 131 cases by Peterson et al. (1983b). This chapter describes a further series of 74 cases.

10.2 METHODS

Clinical material

The case material comprised cats presenting by referral to the author or directly to veterinary surgeons in private practice who agreed to take part in the study. A tentative diagnosis was made on the basis of historical and clinical features. Subsequently, blood samples were collected from conscious animals by jugular or cephalic venepuncture after a minimum of 12 hours' starvation. Potassium EDTA tubes were used for samples for routine haematological examination and plain tubes for samples for biochemistry.

With the exception of thyroid hormone determinations, all other measurements were made within 24 hours of sampling. Serum was stored at -40°C if it was not possible to assay for T4 and T3 within this period.

Haematological tests

The total RBC count was carried out using a one to 50,000 dilution of whole blood in Isoton II, (obtainable from Coulter Electronics Ltd., Cold Harbour Lane, Harpenden, Hertfordshire, England). The total RBCs were counted electronically using a Coulter Electronic Particle Counter, Model ZF6, (obtainable from Coulter Electronics Ltd.), each reported result being the mean of duplicates.

The PCV was determined by the microhaematocrit method using plain capillary tubes and a microhaematocrit centrifuge obtainable from Hawksley & Sons Ltd., 12 Peter Road, Lancing, Sussex, England.

The haemoglobin concentration was measured directly after conversion to cyanmethaemoglobin using a 3.3 mg/ml solution of potassium cyanide (Zap-0-Globin, obtainable from Coulter Electronics Ltd.) and read directly in a haemoglobinometer, also obtained from Coulter Electronics.

Total white blood cell counts were performed on the Coulter Counter previously described. Whole blood samples were diluted one to 5,000 with isotonic saline and RBCs were lysed with Zap-0-Globin. For the differential white cell count, blood films were fixed with eosin methylene blue compound (Leishman staining solution, obtainable from BDH Chemicals, Poole, Dorset, England) and 200 cells counted under oil immersion microscopy.

The thrombocyte count was determined by the pre-dilution technique. Whole blood was diluted one to ten with isotonic saline and centrifuged using a Coulter Thrombofuge. The thrombocyte-containing supernatant was then diluted one to 200 with Isoton and counted by a Coulter Thrombocounter C.

Biochemical tests

The serum T4 and T3 concentrations were measured as described in Chapter 6.

Serum AP, ALT, cholesterol, urea and inorganic phosphate concentrations were all measured with the aid of a Shimadzu CL-720 clinical analyser.

The serum AP concentrations were determined at 30°C by a commercially available kit method (Gilchem Single Vial Reagent System ALP, obtainable from McQuilkin & Co., 21 Polmadie Avenue, Glasgow, Scotland). The technique involves the hydrolysis of p-nitrophenylphosphate to p-nitrophenol and inorganic phosphate by the AP in the test sample. The rate of p-nitrophenol formation is directly proportional to AP activity and is determined by measuring the rate of change of absorbance at 405 nm. At low, medium and high levels of AP activity, the within-assay cvs were 7.4, 3.2 and 2.1 per cent and the corresponding figures for between-assay cvs were 7.4, 7.7 and 9.3 per cent respectively.

The serum ALT concentrations were determined at 30°C by a commercially available kit method (Gilchem Single Vial Reagent System ALT), obtained as described previously. ALT in the sample catalyses the combination of alpha-oxoglutarate and L-alanine to L-glutamate and pyruvate. The latter subsequently oxidises nicotinamide adenine dinucleotide (reduced) (NADH) to nicotinamide

adenine dinucleotide (NAD) with the formation of L-lactate. Lactate dehydrogenase is included in the reagent to prevent interference from endogenous pyruvate which is normally present in serum samples at low concentrations. Oxidation of NADH causes a decrease in absorbance at 340 nm and the rate of change of absorbance is directly proportional to the ALT activity in the serum. At low and high levels of ALT activity, the within-assay cvs were 8.9 and 1.6 per cent and the corresponding figures for between-assay cvs were 10.3 and 3.2 per cent respectively.

The serum total cholesterol concentrations were determined by a commercially available kit method (Gilchem Single Vial Reagent System Cholesterol), obtainable as described previously. The technique involves the hydrolysis of cholesterol esters in the serum into free cholesterol and fatty acids, by means of cholesterol esterase. The free cholesterol is then oxidised to cholesten-3-one and hydrogen peroxide in the presence of cholesterol oxidase. 2-4 Dichlorophenol and 4-aminoantipyrine then combine with the hydrogen peroxide in the presence of peroxidase to produce red quinoneimine. The intensity of the colour thus produced measured spectrophotometrically at 500 nm is directly proportional to the total cholesterol concentration of the sample. For low and high concentration controls, the mean within-assay cvs were 1.5 per cent and 0.9 per cent, with the corresponding between-assay cvs being 1.5 per cent and 1.2 per cent respectively.

The serum urea concentrations were determined at 30°C using a modification of a commercially available kit method for blood urea nitrogen (BUN) (Gilchem Single Vial Reagent System BUN), obtainable as described previously. The technique involves the hydrolysis of

urea in the sample to ammonia and water, catalysed by urease. The ammonia thus produced combines with 2-oxoglutarate in the presence of NADH during the glutamate dehydrogenase catalysed synthetic reaction, to yield glutamate. An equimolar quantity of NADH undergoes oxidation during the reaction and thus the decrease in absorbance at 340 nm is directly proportional to the blood urea in the sample, when the BUN standards supplied are replaced by pure urea standards. Using serum urea controls, at low and high concentrations of urea activity, the within-assay cvs were 2.6 and 4.5 per cent and the corresponding figures for between-assay cvs were 8.0 and 3.2 per cent respectively.

The serum inorganic phosphorus concentrations were determined by a commercially available kit method (Gilchem Diagnostics Inorganic Phosphorus), obtained as described previously. The technique involves the reaction of inorganic phosphorous in the sample with ammonium molybdate in an acid medium to form a phosphomolybdate complex which absorbs light at 360 nm. This absorbance is directly proportional to the amount of inorganic phosphorus present in the sample. At low and high inorganic phosphorus concentrations, the within-assay cvs were 5.1 per cent and 6.4 per cent and the corresponding figures for between-assay cvs were 4.5 per cent and 1.8 per cent respectively.

The serum calcium concentrations were determined by atomic absorption spectroscopy using an IL 357 Atomic Absorption Spectrometer, supplied by Allied Instrumentation Laboratory, Kelvin Close, Birchwood Science Park, Warrington, Lancashire, England. Samples were diluted with 0.1 per cent calcium chloride and compared with calcium nitrate standards. Within and between-assay cvs were not available for the technique.

Total and individual serum protein concentrations were determined by the biuret method and by agarose gel electrophoresis as described in Section 4.4.

Radiography

Lateral and dorsoventral thoracic radiographs of conscious patients were taken using a Siemens Heliophos 4E X-ray machine supplied by Siemens Ltd., Wheatfield House, 6 Wheatfield Street, Edinburgh, Scotland. They were recorded on Kodak Ortho G X-ray film (Kodak Ltd., P.O. Box 10, Dallimore Road, Manchester, England). Processing was carried out automatically in a Cronex X-ray Film Processor, (Du Pont (UK) Hawkesden Road, St. Neots, Huntingdon, Cambridgeshire, England) at a developer temperature of 32°C and a dryer temperature of 30°C.

Electrocardiography

Recordings from the six basic limb leads (I, II, III, aVR, aVL and aVF) were recorded from conscious individuals using a Cardiofax 3-channel ECG recorder, manufactured by Nihon Kohden and supplied by Holstar Instruments Ltd., Haywards Heath, Sussex, England.

Treatment

Confirmation of the diagnosis was by the demonstration of elevated serum concentrations of total T3 and/or T4. Unless the owners refused, treatment of all cases was by surgical thyroidectomy.

Pre-operative treatment. All cases were treated three times daily for a minimum of one week with 10 mg aqueous potassium iodide solution (10 mg/ml) in an attempt to reduce the circulating T4 and T3 concentrations and the vascularity of the thyroid gland. This solution was readily taken by most cats when mixed with food. Inappetent or anorexic animals were drenched with

the solution using a syringe. Excessive salivation was not noted as a result of either route of administration. In addition, propranolol (Inderal, Imperial Chemical Industries) was administered at a dosage of 2.5 mg three times daily to reduce the heart rate. This dosage was increased to 5 mg three times daily if the heart rate remained above 200 beats per minute after three days' treatment.

In some animals during this period, serum samples were collected as described previously to monitor T4 and T3 concentrations.

Anaesthesia. All cats were premedicated with acetylpromazine (ACP injection 2 mg/ml, C-Vet) at a dose of 0.05 mg/kg bodyweight. Anaesthesia was induced using intravenous ketamine hydrochloride (Parke, Davis) at a dose of 25 mg/kg bodyweight, and maintained with a halothane (Fluothane, Imperial Chemical Industries) and nitrous oxide mixture administered via an Ayres T piece and cuffed endotracheal tube in a non-rebreathing system, using high flow rates (three times the minute volume).

Surgical technique. The patients were placed in dorsal recumbency, with the neck extended and the legs pulled caudally. A ventral, midline skin incision was made from the larynx to the manubrium. After blunt dissection of subcutaneous fascia, the sternohyoideus and sternothyroideus muscles were separated in the midline.

The thyroid lobes and cranial parathyroid glands were identified and inspected. As pre-operative thyroid scanning facilities were not available, the decision as to whether to perform a unilateral or bilateral thyroidectomy was a clinical one based on

the size and appearance of the thyroid lobes. In all but one case (110989) thyroidectomy was by the extracapsular technique, although efforts were always made to preserve the cranial parathyroid gland of affected lobes whether there was unilateral or bilateral involvement. The caudal thyroid vasculature was identified and ligated using three metric chromic catgut. The cranial parathyroid gland was dissected free of the thyroid lobe using fine scissors, taking care to avoid damaging its vasculature. The cranial thyroid vessels were ligated with three metric chromic catgut and the gland subsequently removed by blunt dissection. Throughout the procedure, it was necessary to maintain meticulous haemostasis to allow visualisation of the cranial parathyroid gland. The incision was then closed routinely.

Where involvement of the other thyroid lobe was obvious, the above procedure was repeated.

In one case of bilateral lobe involvement, intracapsular thyroidectomy was carried out. After exposing the thyroparathyroid glands and ligation of the caudal thyroid vein of one abnormal lobe as described above, a small nick incision was made in an avascular area of the ventrocaudal aspect of the thyroid capsule. The incision was extended cranially using small scissors. The thyroid parenchyma was bluntly dissected off the thyroid capsule using sterile cotton buds taking care not to place any tension on the cranial thyroid vessels. Where thyroid tissue fragments were left attached to the capsule, these were carefully removed even if this required partial capsulectomy. Otherwise, the capsule and the cranial parathyroid gland were left intact. The procedure was then repeated for the other thyroid lobe.

In cases in which thyroid antibody studies were to be carried out, excised lobes were divided. One piece was snap-frozen in liquid nitrogen (see Chapter 14). The rest of the excised tissue was preserved in ten per cent formal saline, prior to histological examination. In the remainder of the cases, the lobes were placed entire into the formal saline.

After formalin fixation, excised thyroid tissue was processed to paraffin and 4u to 5u sections were routinely stained with haematoxylin and eosin.

In cases where both thyroid lobes had been removed, serum calcium and phosphate concentrations were measured 16 hours after completion of surgery and subsequently daily for a further four days. Cases were discharged from the hospital between the fourth and the seventh day post-surgery.

Cases were reassessed at intervals as required after surgery. Initially, owners of unilaterally thyroidectomised animals were warned of the possibility of subsequent involvement of the remaining lobe with recurrence of thyrotoxicosis. With recurrence of the condition in a small number of bilaterally thyroidectomised animals, this warning was extended to all cases.

Medical management. The remaining cases were treated medically using PTU (Propylthiouracil B.P.). The drug was administered at a dose of 50 mg three times daily for three weeks and then reduced to 50 mg twice daily.

10.3 RESULTS

The study comprised 74 cats.

Pre-treatment data

Breed, sex and age. These data are presented in Table 10.1.

There were no pedigree cats in the series. There were more females than males but there was no significant difference in the mean age at presentation between the sexes. The two cats in the series under nine years of age when presented (six years and 7.5 years) were both neutered females.

Historical and clinical features. Fifty-three owners were able to report reasonably accurately the duration of symptoms in their animals before first presentation. This ranged from two weeks to 1.5 years with a mean of 4.0 months. However, many owners reported the onset of signs to be insidious and gauged the time of their first impression of them retrospectively. In five cases, no systemic signs had been noted, these having been presented for annual vaccination, annual health check, feline leukaemia virus testing, an ingrowing claw and oral haemorrhage (one case each).

Table 10.2 lists the frequency of the major historical and clinical features of the disease in the series. Weight loss together with a ravenous appetite were the most common signs. A typical case is shown in Fig. 10.1. Twenty-five per cent of the cats vomited occasionally, usually after eating. One cat vomited regularly each evening after feeding and one vomited hair occasionally. Almost half of the cats had diarrhoea, the faeces varying in consistency from liquid to pale and pasty with macroscopic evidence of steatorrhoea. One animal was faecally incontinent.

Polyuria and polydipsia were present in three quarters of all cases. Three owners had noticed haematuria and two cats were actually presented with this as the primary complaint. One third of the cats showed some respiratory abnormality, usually tachypnoea and panting but two cats were dyspnoeic and two showed episodes of sneezing.

Dermatological signs were also present in one third of the cats. Mats (Fig. 10.2) and local or regional asymmetrical alopecia were the commonest lesions. However, two cats had symmetrical alopecia affecting the caudal thighs and hocks (Fig. 10.3) and the ventral abdomen (Fig. 10.4) in a distribution resembling FEA (see Chapter 12). The remaining haircoat could be more easily epilated in some cases. Some cats had hyperaemia of the skin of the pinnae, possibly reflecting hypertension. An example is shown in Fig. 10.5.

Approximately one half of all cats showed tachycardia (heart rate greater than 240 beats per minute) at rest. Other cardiovascular signs were present in just over one quarter of cats examined. Three cats were presented in congestive cardiac failure, with pleural effusions and muffling of heart sounds. These animals were lethargic and inappetent rather than hyperactive and polyphagic. Three other cats exhibited lethargy and inappetence making a total of six cases (eight per cent) with signs comparable to apathetic hyperthyroidism of man.

Palpable goitre was present in 71 of the 74 cats (96 per cent). Of these, 46 cases (64.8 per cent) were judged to be unilateral and 25 cases (35.2 per cent) bilateral. Of the animals with unilateral goitre, 58.6 per cent affected the left lobe and 41.4 per cent the right lobe. A case of left-sided thyroid goitre

is shown in Fig. 10.6. Thyroid enlargement could not be palpated in three of the 74 cases (four per cent).

In most animals, the enlarged lobes were easily mobile, often between the larynx and the thoracic inlet. Sometimes, lobes became retrotracheal making careful palpation essential if goitre was not to be missed.

Radiography. Thoracic radiography was carried out on ten cases. The summary of results reported here is provided courtesy of A.H.M. van den Broek.

Cardiac size was assessed on lateral radiographs by measurements of the maximum apicobasilar length (ABL) at the level of the carina of the trachea and the maximum craniocaudal width (CCW) at right angles to the ABL. A typical lateral thoracic radiograph from a hyperthyroid cat and the position of these measurements is shown in Fig. 10.7a. The results were compared to those obtained from 30 healthy cats using Student's unpaired t test and are summarised in Table 10.3.

Nine of the ten cases had ABL and CCW values which were greater than the respective means of the healthy cats. The ABL was significantly and the CCW was highly significantly increased in hyperthyroid compared with healthy cats.

Electrocardiography. Pre-treatment electrocardiographic data for ten cats are presented in Table 10.4.

The commonest abnormality was an increase in the R wave amplitude in lead II which occurred in eight cases (80 per cent). An example is shown in Fig. 10.8a. Three cats (30 per cent) had tachycardia at rest. Four cats (40 per cent) had marked notching of the QRS complex, suggestive of cardiac enlargement. Four cats had increased P wave voltages in lead II.

Conduction disturbances included two cats (20 per cent) with left anterior fascicular block and one (ten per cent) with ventricular premature beats (Fig. 10.8a).

Serum thyroid hormone concentrations. Serum total T4 concentrations were determined in all cases but were reported simply as greater than 200 nmol/l in two individuals. Serum total T3 concentrations were determined in 71 cases.

The zero, 25th, 50th, 75th and 100th sample percentiles for these analytes are presented in Table 10.5. The T4 concentrations were greater than three sds from the mean of the reference range in all cases. Serum T3 concentrations were normal in six individuals (mean 1.08 nmol/l, range 0.90 to 1.27 nmol/l). In none of these cats did the serum T4 concentration exceed 80 nmol/l (mean value 70.0 nmol/l, range 63.0 to 80 nmol/l).

Individual T4 and T3 values were highly significantly correlated (Kendall rank correlation, $\tau = 0.68$, $P < 0.001$). This relationship is shown in Fig. 10.9.

Haematological tests. Pre-treatment haematological parameters were measured in 12 cases. These data are presented in Table 10.6.

The RBC count was normal in all cats. The PCV and haemoglobin concentrations were elevated in one (8.3 per cent) and two cases (16.7 per cent) respectively, in one case concurrently. Eight cats had a leucocytosis, which was always due to a neutrophilia. In three cases, this could be explained by pathology unrelated to the hyperthyroidism (one case each of mural endocarditis, periodontal disease and infected end-stage kidney disease). There was a lymphopenia in one case (possibly due to

stress) and a monocytosis and eosinophilia in four and three cases respectively.

Non-specific biochemical tests. Pre-treatment biochemical parameters other than T4 and T3 concentrations were measured in 12 cases. These data are presented in Table 10.7.

Seven cases (58 per cent) showed elevated serum AP concentrations while five (42 per cent) were within the normal range. Serum ALT concentrations were raised in 11 cases (92 per cent) and normal in one case (eight per cent). The serum urea concentration was measured in ten cases, being elevated in seven (70 per cent). The serum cholesterol, calcium and inorganic phosphate concentrations were within the respective normal ranges in all cases tested. The serum protein concentrations showed no obvious trends.

Treatment data. Four cats died before treatment could be undertaken. Six cats were euthanased at the owner's request. Three animals were lost to follow-up after the initial diagnosis and two owners elected not to have their animals treated. Fifty-five animals were treated surgically and four medically.

Of the three cases in which thyroid enlargement was not palpable, one died before surgery could be carried out, one was treated medically and one was lost to follow-up.

Of the cats with apathetic hyperthyroidism, two were euthanased at the owner's request, two were treated surgically and two were treated medically but were lost to follow-up.

Surgical management

Pre-operative management. All cats undergoing surgery were treated for at least seven days prior to surgery with potassium iodide and propranolol as described in Section 10.2. Serum total T4

and T3 concentrations were sequentially monitored during this period in six cases. These data are presented in Table 10.8.

Using potassium iodide and propranolol, in only one case (134562) were serum T4 and T3 concentrations reduced to the euthyroid range. In one other (131335), the T3 concentration returned to the euthyroid range but the T4 concentration remained marginally elevated. Both the T4 and T3 concentrations fell from their initial values in one cat (113897) but still remained grossly elevated. In two cases (127281 and 137264) there was an initial marginal decrease in both T4 and T3 concentrations but these began to rise again before surgery. Thyroid hormone concentrations remained unaffected in case 134770.

In all cats, the thyroid gland became palpably firmer and the heart rates decreased during this period.

Surgery. Affected thyroid lobes varied in size from 1.5 cm to 3.5 cm in length and were often irregular in contour. They ranged in colour from yellow to yellow-green, through tan to dark brown. A typical gland from a case of unilateral disease is shown in Fig. 10.10. Small cysts were frequently present on the surface of the glands, extending into the deeper tissue. The cut surface of a typical gland is shown in Fig. 10.11.

In cases of unilateral lobe involvement, the contralateral lobe appeared atrophic.

Of the 55 animals treated surgically, 39 (70.9 per cent) underwent unilateral and 16 (29.1 per cent) bilateral thyroidectomy. All animals survived the operative procedure. Two died immediately afterwards, probably as a result of cardiac arrhythmias. Both of these had had unilateral lobe removal.

Post-operative management. No further potassium iodide was given post-surgery but propranolol was continued at a dose of 2.5 mg three times daily for a further three days. T4 was not routinely administered post-operatively to either unilaterally or bilaterally thyroidectomised cases.

In the majority of cases from which both thyroid lobes had been removed, the serum calcium concentration fell on the second day post-operatively to values of between 1.8 nmol/l and 2.0 nmol/l. No treatment was administered unless such individuals showed physical signs of hypocalcaemia (muscle tremors, weakness, tetanic spasms, convulsions), when 1.5 ml/kg ten per cent calcium borogluconate (diluted from Calcium Borogluconate 20%, Crown Chemicals) was administered intravenously. Subsequently, intravenous or subcutaneous therapy was given to effect, together with daily maintenance doses of 0.2 g dihydrotachysterol (A.T. 10, Sterling Research Laboratories) or tablets containing calcium lactate (300 mg), calcium phosphate (150 mg) and calciferol (0.0125 mg) (Calcium with Vitamin D Tablets, B.P. (Vet), Veterinary Drug Co.).

Signs of hypocalcaemia developed in seven cats which had undergone bilateral thyroidectomy. In all cases, this occurred between the first and the third day post-operatively. Despite the treatment outlined above, six of the affected animals died, one on the first, two on the second, one on the third, one on the sixth and one on the seventh days post-operatively. Two of these cases were the most severely cachectic in the series. The remaining animal was maintained on Calcium with Vitamin D tablets (one twice daily) for three weeks and remained normocalcaemic when these were subsequently withdrawn.

No other post-operative complications were noted. Thus, 37 of the 39 cases (95 per cent) of unilateral lobe involvement and ten of the 16 cases (63 per cent) of bilateral lobe involvement were treated successfully by surgery.

In order to determine how quickly serum thyroid hormone concentrations returned to normal after surgery, blood samples were collected at varying intervals post-operatively in six cats. The results of this study are shown in Table 10.8.

In four of the six cases, the serum total T4 concentration was within the normal range by the day following surgery and in all cases by the second post-operative day. The serum total T3 concentration was in the normal range in all cases by the day following surgery.

Thyroid histology. Excised thyroid tissue from 54 cases was examined.

In all cases, the histological appearance was compatible with a diagnosis of follicular adenomatous hyperplasia with varying degrees of cystic formation or colloid accumulation. Fig. 10.12 is an example of normal feline thyroid tissue. A typical example of adenomatous hyperplasia is shown in Fig. 10.13. No attempt was made to distinguish between this and true adenoma formation. The degree of replacement of normal thyroid tissue varied from mild to severe. Varying degrees of encapsulation of abnormal tissue were noted (Fig. 10.14).

Lymphocytic infiltrates were observed in 22 cases (41.5 per cent) (Fig. 10.15). While they were found in equal numbers of unilateral and bilateral goitre (11 cases each), bilateral cases of goitre made up only 33 per cent (18 cases) from which thyroid tissue

was examined histologically. Lymphocytes tended to be distributed either paravascularly (Fig. 10.16) or adjacent to relatively normal peripheral thyroidal acini (Figs. 10.17(a) and (b)).

Post-mortem examinations. Post-mortem examinations were carried out on three cases, all of which died before treatment could be carried out.

All three cats were thin. One (140802) had 90 ml of fluid with the characteristics of a modified transudate in the thoracic cavity and 100 ml of the same type of fluid were present in the abdominal cavity. The heart of this cat showed left auricular and ventricular dilatations. The heart of one of the other cats (135619) was flaccid and showed dilatation of the left ventricle while that of the third (109404) had a left ventricular mural endocarditis which on culture yielded occasional colonies of Pasteurella multocida. In the latter, infected emboli were present in the brain, kidney and liver. This cat had pulmonary oedema and the other two, pulmonary congestion.

One cat (135619) had a pale liver with cystic foci. Histologically, there were areas of telangiectasis and an unusual, portal-bridging, fatty, degenerative change.

Two cats had firm, irregular kidneys. Histologically, one (135619) had widespread glomerulonephropathy with protein deposits within the Bowman's capsule and early thickening of the glomerular tufts, and in one kidney, localised pyelitis. This cat had a serum urea concentration of 74.8 mmol/l. The other (140802) had histological evidence of low-grade interstitial nephritis and a blood urea concentration of 32.8 mmol/l.

The stomach of cat 135619 was contracted and apparently atrophic with no obvious rugae. There were multiple, cream, nodular formations around the cardia and some larger, solitary nodules in the body region. Histological sections of these areas confirmed that they consisted of non-neoplastic, lymphoid infiltrations. This cat had enlarged adrenal glands. Histologically, all layers of the adrenal cortex were hyperplastic but this change was most marked in the zona reticularis.

The cat showing bacterial endocarditis also had macroscopic and histological evidence of chronic pancreatitis.

Post-operative follow-up

Clinical findings. Post-operatively, the signs of hyperthyroidism resolved in all cases successfully treated.

Hyperactivity ceased within four days. Some animals subsequently became mildly to moderately lethargic for up to eight weeks following surgery, while others, according to their owners, were normally active. One (110989) slept continually, apart from feeding periods, from two weeks following bilateral thyroidectomy. This animal developed the same bilaterally symmetrical pinna changes (alopecia, hyperpigmentation) as did two experimental hypothyroid cats, (discussed in detail in Chapter 11). Pinna changes were first obvious 17 weeks post-surgery. T4 and T3 determinations confirmed persistent hypothyroidism (Table 10.9). The ear lesions, together with the lethargy, resolved on introduction of T4 therapy (Eltroxin, Glaxo) at a dose of 50 ug twice daily.

Polyphagia tended to persist until normal weight was re-established, when the appetite reverted to normal. The time

required to re-establish normal weight varied from four to 12 weeks in mildly and severely affected animals respectively. One 16-year-old cat increased in bodyweight from 1.8 kg pre-operatively to 4.8 kg within six weeks.

Diarrhoea (but not vomition) continued in a number of cats for up to 12 weeks and appeared to be related to the persistence of polyphagia, resolving when the animals' appetites reverted to normal.

Radiography. Thoracic radiography was carried out in seven cats between seven and 11 weeks (mean ten weeks) after successful surgical treatment of hyperthyroidism. Cardiac size was measured as reported previously. These data are presented and compared with pre-treatment values in Table 10.3 using Student's paired t test.

There were no significant differences between the groups. However, in some cases, reductions in cardiac size were obvious radiographically. An example is shown in Fig. 10.7(b).

Electrocardiography. Post-treatment ECGs were available for six cats, measured between three and 18 weeks (mean 11.3 weeks) after thyroidectomy. These data are presented in Table 10.4. In one cat (140363), clinical and biochemical evidence of hyperthyroidism recurred rapidly after surgery and its ECG parameters did not alter or worsened in the eight weeks between the two measurements. This animal is therefore disregarded when considering ECG alterations after successful therapy.

Of the four animals which had elevated pre-treatment R wave voltages, three (75 per cent) reverted to normal after thyroidectomy. There was no significant change in the fourth despite resolution of the thyrotoxicosis. The notching of the QRS

complex, the left anterior fascicular block and the ventricular premature beats resolved in all affected cats.

Fig. 10.8(b) shows a post-treatment ECG and compares it to a pre-treatment ECG from the same animal (Fig. 10.8(a)).

Serum thyroid hormone concentrations. Serum total T4 and T3 concentrations were monitored serially post-operatively in eight cats. Five of these had had unilateral and three bilateral thyroid lobe excisions. These data are presented in Table 10.9.

The T4 and T3 concentrations of animals with unilateral lobe involvement returned to their normal respective ranges within two to eight weeks post-operatively. In one (140363) the T4 was again elevated five weeks post-surgery, and this cat subsequently developed recurrence of symptoms and signs of hyperthyroidism. Two of the three cases of bilateral lobe involvement also showed a return of T4 and T3 concentrations to their normal respective ranges but this took longer than with the unilateral cases. The third (110989) continued to have non-demonstrable serum total T4 and T3 concentrations 18 weeks after thyroidectomy and received T4 supplementation. Its T4 and T3 concentrations returned to their normal respective ranges and its symptoms and signs of hypothyroidism as previously discussed, subsequently resolved. No other animal in the series of 74 cases required thyroid hormone therapy post-operatively.

Other biochemical and haematological tests. These were not monitored post-operatively.

Recurrence of hyperthyroidism. During a follow-up period of from two to 40 months, hyperthyroidism recurred in four cases in this series. Three of these are discussed in more detail in Chapter 14.

The fourth case (140363) was judged to have unilateral lobe involvement at surgery and was treated by unilateral thyroidectomy. While T4 and T3 concentrations were below the detection limits of the respective assays one week post-surgery, after a further four weeks the T4 concentration was above the upper limit of the reference range (49 nmol/l) although the T3 concentration remained within the normal range (0.9 nmol/l). The cat continued to be restless, extremely aggressive and failed to increase in weight. After a further 11 weeks, the T4 concentration (78 nmol/l) was greater than three sds from the mean of the reference range as determined in Chapter 7 although the T3 concentration (1.2 nmol/l) was at the upper end of the reference range. Further monitoring and prospective surgery were arranged with the owners but the cat was lost to follow-up.

Medical management. Four owners refused surgical treatment on the grounds of cost. Medical treatment was begun using PTU at a dose of 50 mg three times daily. Regrettably, serial monitoring of serum T4 and T3 concentrations together with haematological and biochemical examinations to check for side-effects of drug therapy were also refused because of expense or inconvenience. Treatment was therefore empirical and the maintenance dosage of PTU was 50 mg twice daily.

In all cases, the animals became less restless. While they remained polyphagic, further weight loss ceased but no significant weight gain ensued.

All four cases were lost to follow-up over a period of 14 weeks.

While a considerable amount of information has been published on feline hyperthyroidism since its first description in 1979, only three independent series of ten or more cases have been reported. The study described here is the second largest to be presented.

Pre-treatment data

Breed, sex and age. No pedigree cats were represented in this series. Whether this indicates a reduced incidence of the condition in pedigree cats is unknown as cases have been recorded previously in Siamese, Persians and a Russian blue (Hoenig et al., 1982; Peterson et al., 1982; Peterson et al., 1983b).

Fifty-five per cent of the present series were females but the significance, if any, of this observation is unknown. Other authors have reported that feline hyperthyroidism has no sex predisposition (Peterson, 1982; Hoenig, 1983; Peterson and Becker, 1984). In human medicine, 90 per cent of cases of hyperthyroidism are due to Graves' disease, toxic multinodular goitre or a toxic solitary thyroid nodule and each of these is seen more commonly in females than in males (Toft, Campbell and Seth, 1981). For example, Graves' disease and toxic solitary thyroid nodule are seen approximately seven and six times more commonly respectively in females than in males (Toft, Campbell and Seth, 1981). The slight increase in females to males in the present series is obviously not comparable with such figures.

The mean age at onset in this series was 12.7 years, comparing well with mean ages of 11.8 years for a series of 24 cases (Hoenig et al., 1982), and 12.8 years for a series of 131 cases

(Peterson et al., 1983b). The age range at onset for the present series was six to 18 years. The youngest cases previously recorded were also six years of age (Hoenig et al., 1982; Peterson et al., 1983b). While the mean age at onset of females compared with males in this series was not significantly different, the range was greater for females (six to 18 years) compared with males (nine to 16 years). Previous studies have not subdivided the age at onset of feline thyrotoxicosis according to sex and the significance of these observations is unknown.

Historical and clinical features. The historical and clinical features of the cats in the present series (Table 10.2) compare well with those described in previous reports (Table 3.11). In each, the major symptom was weight loss despite polyphagia. Heat intolerance and intermittent fever were also important features. Thyroid hormones regulate carbohydrate, protein and lipid metabolism and heat production in virtually all body tissues. Increased energy metabolism and heat production from excess thyroid hormone secretion result in the increased appetite, weight loss, muscle wasting, weakness and moderate temperature elevation of thyrotoxicosis (Ingbar and Woeber, 1981).

Hyperexcitability with nervousness and aggressive behaviour are commonly seen in hyperthyroid cats (Holzworth et al., 1980; Hoenig et al., 1982; Peterson et al., 1983b). Thyroid hormones interact with the nervous system producing overall increased sympathetic drive resulting in these symptoms (Rockey and Griep, 1980; Ingbar and Woeber, 1981).

Gastrointestinal symptoms (vomition, diarrhoea, bulky faeces and increased frequency of defaecation) were important features of

this and previous series of hyperthyroid cats. In human thyrotoxicosis, vomiting is a rare but well-documented symptom. The mechanism is unclear but may result from direct action of thyroid hormones on the chemoreceptor trigger zone (Rosenthal, Jones and Lewis, 1976). Rapid overeating in the hyperthyroid cat may also contribute to vomiting (Peterson *et al.*, 1983b). Thyrotoxic people commonly develop intestinal hypermotility resulting in increased frequency of defaecation and diarrhoea (Middleton and Morrow, 1971). Malabsorption with increased faecal fat excretion, also develops in approximately 25 per cent of human hyperthyroid patients (Thomas, Caldwell and Greenberger, 1973). The exact causes of the steatorrhoea are unknown but appear to include reduction in pancreatic trypsin secretion (Wiley, Lavigne, Liu and MacGregor, 1978) and excess fat intake due to polyphagia (Kasper, 1970; Thomas, Caldwell and Greenberger, 1973). Pancreatic enzyme and absorption tests and faecal fat analyses were not carried out in the present study.

Polyuria and polydipsia occurred in 75 per cent of cases in the present and in previous series of feline hyperthyroidism. While some cats had concurrent evidence of primary renal disease, most cats with polyuria and polydipsia did not. Similar signs are seen in human thyrotoxicosis but the exact cause is unknown. In some cases, a primary polyuria with secondary polydipsia appears to result from decrease in renal medullary solute concentration (medullary "washout") due to increased total renal blood flow (Cutler, Glatte and Dowling, 1967). Other humans with thyrotoxicosis have normal renal concentrating ability and primary polydipsia appears to result from a hypothalamic disturbance associated with the thyroid disease process (Evered, Hayter and Surveyor, 1972).

In the present series, two cats had haematuria as the primary complaint and another owner reported haematuria as one of several symptoms shown by her animal. Although it is possible that this finding was coincidental, haematuria has been reported in two previous hyperthyroid cats (Jones and Johnstone, 1981; Watson et al., 1981). Bleeding tendencies in untreated human thyrotoxicosis are uncommon. However, an associated thrombocytopenia, reversible by thyroidectomy, has been described (Woodruff, 1940). Prothrombin deficiency may rarely result from hyperthyroid hepatopathy (Bechgaard, 1946). In addition, thyrotoxicosis may lead to depressed activity of several coagulation factors (II, VII, IX and X) although the effects are not usually apparent clinically (Ikkala, Eisalo and Heinivaara, 1962).

No other signs of bleeding tendencies were seen in the cats in the present series. Further investigations into their haematuria were not carried out and the symptom did not recur after successful surgical therapy.

Respiratory abnormalities, chiefly tachypnoea and dyspnoea at rest were seen in 33 per cent of cats. Hoenig et al. (1982) reported respiratory abnormalities in the same percentage of 24 cases and Peterson (1983b) in 25 per cent of 131 cases. In man, thyrotoxicosis produces a decreased vital capacity, decreased pulmonary compliance and increased minute ventilation (Ingbar and Woeber, 1981). These abnormalities in respiratory function probably result from a combination of respiratory muscle weakness and increased carbon dioxide production (Peterson, 1984). Congestive cardiac failure may contribute to the signs in some cats (Peterson, 1984).

Dermatological abnormalities were found in 32 per cent of cases in the present series. Most abnormalities affected the hair (alopecia, mats, harsh, dry coat, increased ease of epilation) and were probably associated with behavioural changes resulting in decreased or increased grooming, together with direct effects of excessive concentrations of thyroid hormones. One cat was presented for an ingrowing claw. Similar changes have been reported in other hyperthyroid cats (Holzworth et al., 1980; Hoenig et al., 1982; Peterson et al., 1983b). Changes in hair texture, partial hair loss and increased nail growth are also common features of human thyrotoxicosis (Freedburg, 1978a).

Tachycardia was present in more than half of the cats in the present series and other cardiac abnormalities (powerful apex beat, murmur, gallop rhythm and arrhythmias) occurred in 28 per cent of cases. Four per cent of cases had congestive cardiac failure. Cardiomegaly was confirmed in a study of ten cats by comparison with 30 healthy animals. This results from hypertrophic cardiomyopathy (Liu, Peterson and Fox, 1984). Electrocardiographic changes included tachycardia, increased R wave amplitude in lead II, ventricular arrhythmias and intraventricular conduction disturbances. These electrocardiographic changes have been reported previously in feline hyperthyroidism (Peterson et al., 1982; Peterson et al., 1983b; Liu, Peterson and Fox, 1984).

The exact pathogenesis of the cardiac abnormalities associated with hyperthyroidism is unclear but appears to involve a combination of direct action of thyroid hormones on the heart, interactions between thyroid hormones and the sympathetic nervous system and cardiac changes that compensate for altered peripheral

tissue function caused by thyrotoxicosis (Ingbar and Woeber, 1981; Peterson et al., 1982).

In this series, signs compatible with the state of apathetic (masked) hyperthyroidism were seen in six cases (eight per cent). This is close to the figure of "approximately ten per cent" of 131 cases reported by Peterson et al. (1983b). Severe cardiac abnormalities (arrhythmias, congestive cardiac failure) were seen in four of these cases. Cardiac abnormalities have been reported previously to be common in cases of apathetic hyperthyroidism (Peterson, 1984).

Palpable thyroid enlargement was present in 71 of the 74 cats (96 per cent) in the present series. In the remaining three cases, the site of the hyperfunctional thyroid tissue was never identified as one case died before surgery could be carried out and post-mortem examination was not allowed, one was treated medically with PTU and one was lost to follow-up.

Of the cases of unilateral goitre, 58.6 per cent affected the left and 41.4 per cent the right lobe. These figures are virtually identical to those published by Peterson et al., (1983b) who found the left lobe affected in 58 per cent and the right in 42 per cent of 36 cases.

Biochemical tests. As in previous series (e.g. Peterson et al., 1983b), serum T4 concentrations were elevated in all cats. In addition, all T4 values were greater than three sds from the reference mean, a value which has been suggested by Turrel et al. (1984) as useful in differentiating hyperthyroid animals from those healthy individuals with T4 concentrations outside the reference range. The maximum T4 concentration determined was 688 nmol/l,

which is about 15 times the upper value of the reference range. These figures compare closely with those of Peterson et al. (1983b) who, in a series of 131 cases, reported the maximum T4 concentration as 696 nmol/l, 14 times the upper limit of their reference range.

The serum T3 concentration was elevated in 65 of the 71 cases in which it was measured, with a maximum value of 10.30 nmol/l, which is about eight times the upper limit of the reference range. These figures are lower than those of Peterson et al. (1983b), who reported a maximum T3 concentration in 131 hyperthyroid cats of 15.4 nmol/l, which was 17 times the upper limit of their reference range.

Six of the hyperthyroid cats in this series had normal serum T3 concentrations. The T4 concentration in each was only marginally raised. One cat died of Pasteurella endocarditis before surgery and one was euthanased because of congestive cardiac failure. A third cat died on the second day post-surgery. Peterson (1982, 1983), Peterson et al. (1983b) and Peterson (1984) have suggested that hyperthyroid cats with normal T3 concentrations are only mildly affected and that such concentrations would increase into the thyrotoxic range if the disorder were allowed to progress. This may indeed be the case in some cats. However, the results of the present study suggest that some affected animals may be manifesting a condition analogous to the state of "T4 toxicosis" in man, in which the serum total T3 concentration is artificially decreased in hyperthyroidism because of concomitant systemic illness. T4 toxicosis has been discussed in detail in Section 9.6.

In the present series, individual T4 and T3 values were, as in euthyroid animals, highly significantly correlated. Such a relationship might have been anticipated but has not been reported previously.

Elevations in serum ALT, AP and urea were the commonest non-specific biochemical abnormalities seen, occurring in 92 per cent, 58 per cent and 70 per cent of cases respectively. Previous reports have discussed the frequency with which ALT and AP are elevated in feline hyperthyroidism (e.g. Peterson, 1983b and fully reviewed in Section 3.11). In human thyrotoxicosis, similar enzyme increases are frequent and are usually attributed to hepatic dysfunction (Dooner, Parada, Aliaga and Hoyl, 1967). The causes of hepatic damage in thyrotoxicosis are unclear and may include malnutrition, congestive cardiac failure, infections, hepatic anoxia and direct toxic effects of thyroid hormones on the liver (Dooner et al., 1967). In human thyrotoxicosis, serum concentrations of the bone isoenzyme of AP are commonly increased (Dooner et al., 1967) and this may result from increased bone turnover.

Elevated serum blood urea concentrations were found in 70 per cent of the cases studied in the present series. This is considerably higher than the 25 per cent of cases reported by Peterson et al. (1983b). The reasons for this discrepancy are unclear.

The serum cholesterol concentration was within the normal range in all cats tested. Similar results have been reported by Holzworth et al. (1980) and Peterson et al. (1983b).

The pre-treatment serum inorganic phosphate concentration was measured in only three cases in the present series but was normal in each. Peterson et al. (1983b) reported that 20 per cent of 131 hyperthyroid cats had increased serum phosphate concentrations. This may result from renal insufficiency. In addition, thyroid hormones stimulate bone resorption directly,

resulting in elevations in serum calcium with concomitant decreases in serum parathyroid hormone concentrations. This causes increased tubular resorption of phosphate, which together with the increased phosphate loads from bone resorption and muscle catabolism, result in hyperphosphataemia (Mosekilde and Christensen, 1977; Parfitt and Kleerekoper, 1980).

Haematological tests. In the present study, there were minimal changes in the red cell series of 12 cats studied, with elevations of PCV and haemoglobin in one and two cases respectively. None of the cats was anaemic. In a series of 131 cases, Peterson et al. (1983b) reported increases in PCV, RBC and haemoglobin concentration in 45 per cent, 14 per cent and 14 per cent respectively. In addition "a small number" of cats with severe thyrotoxicosis developed mild anaemia.

The erythrocytosis of hyperthyroidism appears to result from the direct effect of thyroid hormones, via beta-adrenergic receptors, on erythroid marrow as well as by increased production of erythropoietin (Das, Mukherjee, Sarkar, Dash and Rastogi, 1975; Popovic, Brown and Adamson, 1977). By increasing the rate of RBC differentiation and decreasing total RBC maturation time, erythropoietin excess may cause release of macrocytes into the circulation (Peschle, 1980). Anaemia is also encountered occasionally in human thyrotoxic patients but the cause is unknown (Rivlin and Wagner, 1969).

Treatment data

Surgical management

Pre-operative management. All cats were treated prior to surgery with a combination of propranolol and potassium iodide.

This treatment was effective in slowing the elevated heart rates but, in all but two cases, ineffective in lowering serum thyroid hormone concentrations to the euthyroid range.

Propranolol slows heart rate by beta-adrenergic blockade (Peterson, 1982; Hoenig, 1983; Peterson, 1983). It is generally considered to have no effect on serum thyroid hormone concentrations of either hyperthyroid people (Toft, Campbell and Seth, 1981) or cats (Peterson, 1982; Hoenig, 1983; Peterson, 1983). However, in human medicine, several clinical investigations conducted in euthyroid, hyperthyroid and T4-treated hypothyroid people have shown a decrease in the serum T3 concentrations with a concurrent increase in the serum rT3 concentrations during propranolol treatment, which was reversible when such therapy was withdrawn (Lotti, Delitala and Devilla, 1977; Wiersinga and Touber, 1977). The thyroid hormone metabolism of euthyroid dogs is not altered by propranolol (Center, Mitchell, Nachreiner, Concannon and Reimers, 1984).

Large doses of stable iodine are reported to block T4 and T3 release from the human thyroid gland and to lower serum thyroid hormone concentrations (McKenzie, Zakarija and Bonnyns, 1979; Ingbar and Woeber, 1981). However, other authors report that serum thyroid hormone concentrations are not affected by iodine (Toft, Campbell and Seth, 1981).

Recently, the combination of propranolol and potassium iodide, unlike the use of either drug alone, has been reported to cause a fall in plasma total T4 and T3 to the normal range in most human hyperthyroid patients (Feek, Sawyers, Irvine, Beckett, Ratcliffe and Toft, 1980). Similar changes in plasma thyroid

hormone concentrations do not appear to occur in most hyperthyroid cats.

The thyroid lobes of cats treated with potassium iodide were palpably firmer than prior to therapy. In human medicine, potassium iodide is used routinely for ten days prior to thyroidectomy to reduce the size and vascularity of the thyroid gland (Toft, Campbell and Seth, 1981). Pre-operative potassium iodide therapy of hyperthyroid cats is, therefore, of benefit even in the absence of a reduction in plasma thyroid hormone concentrations.

While Hoenig (1983) has recommended the administration of saturated potassium iodide solution to hyperthyroid cats, it is known that concentrated solutions may result in vomiting due to gastric irritation (Thoday, 1986). Potassium iodide was therefore administered to these cats as a 10 mg/ml aqueous solution. Black and Peterson (1983) have reported that potassium iodide has a brassy taste which may result in partial to complete anorexia in some cats. No such difficulties were encountered in the present series. Indeed, the author can vouch for the fact that the preparation used was completely tasteless.

Anaesthesia and surgical technique. In the present series, cats were premedicated with acetylpromazine. Atropine was not used as it is known to cause sinus tachycardia or to enhance anaesthetic-induced cardiac arrhythmias (Black and Peterson, 1983). Anaesthesia was induced using ketamine hydrochloride and maintained with a halothane and nitrous oxide mixture. This anaesthetic regime has been recommended for use in hyperthyroid cats (Birchard, Peterson and Jacobson, 1984).

In this series, 70.9 per cent of cases were, on macroscopic examination at surgery, judged to have unilateral thyroid lobe involvement and 29.1 per cent bilateral lobe involvement. This is the reverse of a series of 131 cases reported by Peterson et al. (1983b) who, with the aid of thyroid imaging techniques, found unilateral lobe involvement in 29 per cent of cases and bilateral involvement in 71 per cent of cases. Hoenig et al. (1982), also aided by thyroid imaging studies, reported bilateral lobe involvement in 79 per cent of affected cats.

The reason for such a marked difference in these series is not clear. While it is tempting to speculate that thyroid imaging in the present series would have led to the diagnosis of a greater number of cases of bilateral lobe involvement, this is unlikely as recurrence of hyperthyroidism took place in only two of the unilaterally thyroidectomised cases. At the time of writing, the period of follow-up in these cases has varied from two to 40 months.

It is, of course, possible that recurrence may still follow in some of the unilateral cases in due course. The possible reasons for recurrence in bilaterally thyroidectomised cases are discussed in Chapter 14.

With the exception of one case, all thyroidal excisions were by the extracapsular technique. This method was used to prevent small fragments of thyroid tissue remaining attached to the capsule because of the resultant risk of recurrence of hyperthyroidism due to continued hyperplasia (see Chapter 14). However, the technique carries a moderate risk of damage to the cranial parathyroid gland or to its blood supply with resultant hypercalcaemia in cases of bilateral lobe excision (Peterson, Birchard and Mehlhaff, 1984).

In the present series, hypocalcaemia developed in seven of the bilaterally thyroidectomised cats. Despite intensive therapy, six of these animals died. Intracapsular excision is likely to have reduced the number of deaths due to hypocalcaemia but increased the cases of recurrent hyperthyroidism. In human medicine, further surgery for recurrent hyperthyroidism combines technical difficulties and unacceptable morbidity (Toft, Campbell and Seth, 1981) and may cause technical difficulties in cats. Facilities for radioiodine therapy were not available to the author. Medical therapy using PTU may result in mild to serious drug associated reactions (Peterson, 1981, 1982; Peterson, Cavanagh and Hurvitz, 1983; Peterson et al., 1984). In addition, in this series, owner compliance with PTU therapy was poor with all four such treated cases being lost to follow-up within 14 weeks of beginning therapy.

One possible way of circumventing the problems outlined above is to carry out intracapsular thyroidectomy followed by subsequent excision of the caudal two thirds of the thyroid capsule. This would remove the majority of thyroid remnants without disturbing the vasculature to the cranial parathyroid gland. This approach has been used in a small number of cases of hyperthyroidism (not reported here) and results are encouraging.

Thyroid histology. The histological findings in this series are in general agreement with other reports (Peterson et al., 1982; Peterson et al., 1983b; Birchard, Peterson and Jacobson, 1984; Peterson, 1984; Peterson and Becker, 1984). However, there have been no previous reports of lymphocytic infiltrates in hyperfunctional thyroid tissue. Such infiltrates are closely

correlated with the presence of serum thyroid autoantibodies in human thyroid disease (Schade, Owen, Smart and Hall, 1960) and investigations into autoantibody production in feline hyperthyroidism were subsequently carried out. The results are reported in Chapter 14.

No cases of functional thyroid carcinoma were identified in the present series. However, this would not be unexpected in a series of 54 thyroid histological examinations, as Peterson et al. (1983b) reported that the prevalence of functional thyroid carcinoma in cats is only one to two per cent.

Post-mortem examinations. The pathological changes found at post-mortem examination of three of the cats in this series are consistent with those reported previously.

One cat had congestive cardiac failure and the remaining two had cardiac changes similar to those reported previously (Jacobs, Hutson, Dougherty and Kirmayer, 1986). There is clinical and experimental evidence in man and dogs that hyperthyroidism may result in congestive heart failure in the absence of antecedent cardiovascular disease (Paitnek-Leinissen and Olsen, 1967; Shapiro, Steier and Dimich, 1975). One cat had left ventricular mural endocarditis with widespread infected emboli formation. This change has not been reported previously in feline hyperthyroidism.

Hepatic fatty change occurred in one cat. This has been reported previously in feline thyrotoxicosis (Jones and Johnstone, 1981).

One cat had diffuse adrenocortical hyperplasia. Liu, Fox and Peterson (1984) reported that eight of 23 hyperthyroid cats showed nodular hyperplasia and three, diffuse hyperplasia of zones

of the adrenal cortex. However, these involved the zona glomerulosa and the zona fasciculata while the most marked changes in the present case were found in the zona reticularis.

Adrenocortical hyperplasia is an uncommon finding in cats (Liu, Fox and Peterson, 1984). In human patients with thyrotoxicosis, thyroid hormone excess increases the metabolic clearance rates of cortisol and aldosterone, which in turn lead to an increase in their secretion rates (Peterson, 1958). Adrenocortical hyperplasia in hyperthyroidism may represent a compensatory response to maintain circulating concentrations of adrenocortical steroids within their normal ranges (Peterson, 1958), although it is possible that it is simply the end result of a chronic response to stress produced by the disease.

One cat had widespread glomerulonephropathy, and gastric atrophy with prominent gastric lymphoid infiltrations. Because these changes may be commensurate with altered immune status or autoimmune disease, it would have been interesting to carry out serum thyroid antibody and ANA determinations in this cat but, regrettably, no serum samples remained to be included in the study reported in Chapter 14.

Post-operative follow-up

Clinical findings. In all cases treated successfully, the signs of hyperthyroidism resolved. Hyperactivity was the first sign to disappear and this correlated with the rapid return to normal of serum thyroid hormone concentrations. Some cats subsequently became lethargic while others remained normally active. This appeared to correlate well with the rate at which thyroid hormone concentrations returned to the normal range (discussed below).

It is interesting that polyphagia tended to persist until animals regained their normal bodyweight and then resolved. This mechanism is obviously independent of serum thyroid hormone concentrations. The persistence of post-operative diarrhoea was linked to that of polyphagia, suggesting that the latter was the cause of the former.

Radiographic and electrocardiographic changes. While significant reductions in cardiac size were not recorded on statistical comparison of pre- and post-treatment radiographic measurements of seven hyperthyroid cats, some individual cases showed obvious improvements post-surgery. Follow-up radiographs were taken between seven and 11 weeks after surgery and it is possible that if this period had been extended, significant reductions in cardiac size might have been noted. ECG abnormalities also improved or resolved in successfully treated cases. Previous authors have noted that effective therapy of feline hyperthyroidism may produce resolution of the ECG changes (Peterson et al., 1982) and cardiomyopathy (Liu, Peterson and Fox, 1984) commonly associated with the disease.

Serum thyroid hormone concentrations. In all of the six cases in which T3 and T4 concentrations were monitored serially post-operatively, these values returned to their normal ranges within 24 and 48 hours respectively. These findings agree with those of Peterson, Becker and Hurley (1980), Peterson (1982, 1983) and Birchard, Peterson and Jacobson (1984).

The serum thyroid hormone concentrations of eight cats treated by surgical thyroidectomy were monitored serially for between five and 32 weeks post-surgery. In all cases which had

undergone unilateral thyroidectomy, the remaining thyroid lobe returned to normal function within eight weeks. Of the three cases of bilateral thyroidectomy, two had serum T4 concentrations in the normal range within 14 weeks. Similar findings have been reported by Peterson (1982), Black and Peterson (1983), Hoenig (1983) and Peterson (1983).

Theran and Holzworth (1980), Olsen (1982) and Martin and Capen (1983) have reported that bilateral thyroidectomy in hyperthyroid cats results in permanent hypothyroidism. This is clearly incorrect. Hoenig et al. (1982) and Birchard, Peterson and Jacobson (1984) reported a return to normal thyroid hormone concentrations between six weeks and six months after bilateral thyroidectomy in hyperthyroid cats. All authors recommend beginning exogenous thyroid hormone supplementation within 24 to 48 hours of bilateral thyroidectomy. This would seem to be contraindicated as exogenous thyroid hormones tend to suppress endogenous thyroid hormone production, delaying a return to T4 and T3 self-sufficiency. For this reason, T4 supplementation was not used in this series until clinical and biochemical signs of persistent hypothyroidism were noted. This occurred in only one case. The possible sources of endogenous thyroid hormone production after bilateral thyroidectomy are discussed in detail in Section 3.11 and Chapter 14.

Other biochemical and the haematological changes found in hyperthyroidism return to normal after successful treatment of the condition (Peterson et al., 1983b). Post-operative studies of these parameters were not carried out in this series.

Medical management. Medical treatment with PTU was only used in this study where owners refused surgery on the grounds of cost. The cases were managed empirically on the same grounds. Peterson (1982, 1984) discussed the problems of owner compliance when feline hyperthyroidism is managed medically. These problems, perhaps caused by the potential expense of necessary haematological and biochemical monitoring, resulted in all four cases being lost to follow-up within 14 weeks of beginning therapy.

11. EXPERIMENTAL PRODUCTION OF FELINE HYPOTHYROIDISM

11.1 INTRODUCTION

No naturally-occurring cases of acquired, feline hypothyroidism have been reported in the veterinary literature. Attempts have been made to induce the condition experimentally, but the clinical and biochemical changes that ensued were not monitored in detail (reviewed in Section 3.12).

This chapter describes the production of feline hypothyroidism in two cats and monitors the clinical and laboratory changes that resulted over a period of 91 weeks.

11.2 EXPERIMENTAL ANIMALS

Two five-year-old, female, domestic short-haired cats (ex-breeding queens) were supplied by the Centre for Laboratory Animals, University of Edinburgh. Immediately on receipt, they were given a detailed physical examination and found to be healthy. They were then vaccinated against feline panleucopenia and feline upper respiratory disease (calici and herpes viruses) using Katavac P and Katavac CH nasal, both supplied by Duphar Veterinary Ltd., Southampton, England.

11.3 ANIMAL ACCOMMODATION

The two cats were accommodated in separate steel cages in the same room. The walls and floor of the room were covered in

heavy grade polythene to guard against contamination by spilled excreta or aerosols. The unit was self-contained with the exception of a central area for preparation of food. Protective clothing (coats, hats, rubber gloves and boots), together with disposable face-masks, were worn by animal attendants until radioactivity levels were judged to have fallen to safe levels (considered to be five per cent of each administered dose). Radiation exposure to staff was monitored by individual thermo-luminescent dosimeters supplied by the National Radiation Protection Board, Harwell, England.

11.4 PHARMACEUTICALS

Radioiodine

^{131}I was obtained as sodium iodide (^{131}I) for injection B.P., with specific activity of 185 MBq (5 mCi) per microgram of iodine at the date and hour stated on the label, and containing sodium thiosulphate as reducing agent. The preparation was obtained from the Radiochemical Centre, Amersham, England. Dilutions were made into sterile, isotonic saline.

Thyroid stimulating hormone

Bovine TSH was obtained in 10 iu vials as Thyrotropar from the Armour Pharmaceutical Company, Hampden Park, Eastbourne, Sussex, England, and donated by courtesy of Berk Pharmaceuticals. The diluent was aqueous sodium chloride solution containing 9 mg sodium chloride/ml without preservative.

11.5 METHODS

Preparation of animals and induction of hypothyroidism

For the first five weeks, the cats were fed on fresh bovine

heart supplied ad libitum and deionised water was given to drink. They were totally deprived of food for a period of three days before and three days after the administration of radioiodine. Subsequently they were fed on a commercially available tinned food (Whiskas, Pedigree Petfoods, Melton Mowbray, England) given ad libitum, and water from the mains supply.

Twenty-four hours and again four hours before the administration of radioiodine, each cat was injected intramuscularly with 10 iu TSH.

To minimise risks to people handling the cats, ^{131}I was administered under light anaesthesia. This was induced using a dose of 33 mg/kg ketamine hydrochloride (Parke, Davis), administered intramuscularly. 1 mCi/kg Na ^{131}I in a total volume of 5 ml was injected into a cephalic vein using a lead-shielded syringe, cat 1 receiving a total dose of 3.8 mCi and cat 2, 3.2 mCi ^{131}I .

The cages were cleaned daily and cleaning cloths, litter and excreta were collected and incinerated. The cats were examined with a Geiger-Müller counter at weekly intervals to estimate ^{131}I retention. Physical examinations and blood sampling were begun 23 days after Na ^{131}I administration.

Monitoring of physical and laboratory parameters

Physical and laboratory parameters were monitored for a five week period (weeks 1-5) prior to thyroid ablation. Apart from a four week period after the administration of ^{131}I , when the cats were deemed to be too radioactive to handle, other physical and the haematological and biochemical parameters were assessed weekly for 23 weeks (9-34) and then at approximately monthly intervals. Although detailed monitoring then ceased, further investigations were carried out intermittently. Full physical examinations were

carried out at each inspection. Blood samples were collected after 24 hours' starvation from conscious animals by jugular venepuncture into potassium EDTA (for cytology) and into tubes without anticoagulant for harvesting serum (for biochemistry).

With the exception of thyroid hormone determinations, all other measurements were made within 24 hours of sampling. Serum was stored at -40°C for the T4 and T3 assays which were carried out in batch mode.

Assessment of physical parameters

Demeanour and appetite were assessed daily and the observations meant to give a weekly result. The cats were fed once daily at 14.00 hours. In an attempt to minimise subjectivity, these observations were scored as shown:

General demeanour score: 1 - Lethargy

2 - Quiet: reduced activity

3 - Normally active

4 - Restless: unwilling to settle
for more than short periods
of time

5 - Continually hyperactive

Appetite score:

1 - Anorexia

2 - Inappetent: significant quantity
of food left daily

3 - Reduced appetite: consuming most
food over 24 hours

4 - Normal appetite: all food eaten
within three hours

5 - Polyphagia: crying for food

6 - Pica

Haematological tests

The RBC count, PCV and haemoglobin concentrations were determined as described in Chapter 10.

The erythrocyte sedimentation rate (ESR) was determined as mm per hour after allowing blood to stand in a Wintrobe haematocrit tube.

To determine the reticulocyte count, five drops of blood were incubated with 2 ml brilliant cresyl blue stain (containing 0.4 g brilliant cresyl blue in an aqueous solution of 20 ml of three per cent sodium citrate solution and 80 ml of 0.85 per cent sodium chloride solution) in a waterbath at 37°C for one hour.

Subsequently, the incubate was spun gently in a Clandon T52.1 centrifuge (obtainable from Clandon Scientific Ltd., Lysons Ave., Ash Vale, Aldershot, Hampshire, England), the supernatant stain decanted and smears prepared. One thousand RBCs were counted under oil immersion microscopy and the number of reticulocytes expressed as a percentage of the total.

Biochemical tests

Serum total T4 and T3 concentrations were determined as described in Chapter 6.

The serum cholesterol concentration was determined using the Gilchem single vial reagent system, as described in Chapter 10.

Creatinine kinase (CK) was measured at 30°C by a commercially available kit method (Gilchem single vial reagent system CK, obtainable from McQuilkin and Co., as described previously). CK catalyses the phosphorylation of adenine diphosphate (ADP) in the presence of creatinine phosphate to form adenine triphosphate (ATP) and creatinine. The auxillary enzyme hexokinase catalyses the phosphorylation of glucose by the ATP formed to produce ADP and

glucose-6-phosphate (G-6-P). The G-6-P is oxidised to phosphogluconate with the concomitant production of NADH. The rate of NADH formation, measured spectrophotometrically at 340 nm is directly proportional to the CK activity in the serum sample. The mean within-assay and between-assay cvs by this technique were 3.8 per cent and 5.6 per cent respectively.

Total and individual serum protein concentrations were determined as described in Chapter 4.

Skin biopsy

Skin biopsies were taken from each cat during week 119.

The cats were premedicated with atropine sulphate (Atropine Sulphate Injection B.P., Veterinary Drug Company), administered subcutaneously. General anaesthesia was induced with five per cent thiopentone sodium (Intraval Sodium, May and Baker) administered intravenously, and maintained by halothane (Fluothane, Imperial Chemical Industries) via a cuffed endotracheal tube. Biopsy sites were the dorsal muzzle, frontal region, right pinna, dorsal mid-thorax, right lateral thorax, right lateral abdomen, dorsal mid-lumbar area, dorsal tail-base, xyphysternum and the left lateral carpus. Hair was removed over these areas using electric clippers but the skin was not cleaned. Full thickness, punch biopsies were taken from all sites using 6 mm, disposable, sterile biopsy punches obtainable from Stiefel Laboratories (UK) Ltd., Wellcroft Road, Slough, England.

Specimens were blotted gently on a dry swab, placed subcutis down on a piece of card and immersed immediately and individually in ten per cent formol saline. Samples were embedded in agar, processed to paraffin and 4 μ to 5 μ sections cut and stained with

haematoxylin and eosin, Martius scarlet blue, PAS reagent, Alcian blue and Verhoeff's haematoxylin.

Electrocardiography

Recordings from the six basic limb leads (I, II, III, aVR, aVL and aVF) were taken from conscious individuals during week 163 using a Cardiofax 3-channel ECG recorder, obtained as described in Chapter 10.

11.6 RESULTS

In this section, weeks 1-5 refer to the period when the cats were on an iodine-deficient diet, week 6 the time of administration of radioiodine, and week 9 the time of recommencing the physical and laboratory observations.

Physical parameters

Skin. Figs. 11.1 and 11.2 show the cats prior to treatment with Na^{131}I .

By week 9, the coat of cat 2 appeared raised, particularly over the dorsum (Fig. 11.3). Mild thinning of the hair was evident over the trunk in both animals by week 15 but this did not progress to alopecia. By week 23, cat 1 showed mild and cat 2 marked, non-pruritic seborrhoea sicca characterised by variably-sized, bran-like scales, which were particularly obvious over the dorsal trunk (Fig. 11.4). This gradually became more extensive and severe during the remainder of the observation period (Figs. 11.5 and 11.6). By week 29, hair over the dorsal trunk in both animals could be more easily epilated than in normal individuals.

Pinnal changes were first observed in week 62 (cat 2) and week 67 (cat 1) (Figs. 11.7 and 11.8) and gradually worsened subsequently. The lesions were bilaterally symmetrical and consisted of alopecia which began around the ear borders, but which rapidly spread to involve the whole of the lateral and medial, distal halves of the pinnae. By weeks 67 (cat 2) and 79 (cat 1), the skin over these areas was brownish-black and had the texture of emery paper (Figs. 11.9 and 11.10). Small pieces of this hyperkeratotic material could be removed with difficulty to leave shiny but dry epidermis.

Cat 1 showed reduced and cat 2, total absence of grooming behaviour from week 17. By week 79, both animals were matted over the dorsum but this only became severe in cat 2 (Fig. 11.11).

Focal areas of alopecia developed over the cranio-lateral aspects of both carpi (Figs. 11.12 and 11.13) (cats 1 and 2), the caudal hocks (Fig. 11.14) (cat 2) and the dorsal and lateral tail-base (cat 1) (Fig. 11.15) by week 111. Thick, bran-like scales were obvious over the tail-base of cat 2. No other pressure points were affected.

Skin changes were always more severe in cat 2 than in cat 1. In both animals, hair removed by electric clippers on a number of occasions from various sites regrew normally.

Both cats developed apparent shortening and broadening of the muzzle after thyroid ablation (Fig. 11.16).

General demeanour. The means of the daily observations are illustrated on a weekly basis in Fig. 11.17 and show that the changes in both cats were very similar. Normal activity was maintained for between five and six weeks after administration of

radioiodine but then decreased rapidly to lethargy by weeks 16 to 17. This continued until week 40 when both cats reverted to normal activity and this was continued unchanged until the end of the observation period.

Appetite. The means of the daily observations are illustrated in Fig. 11.18 and show obvious differences between the two animals. Cat 1 showed a reduction in appetite from week 11 to week 28 but then reverted to normal. The appetite of cat 2 remained unchanged throughout the experiment.

Bodyweight. The results of these observations are illustrated in Fig. 11.19.

Cat 1 showed some weight increase until administration of the radioiodine. Subsequently there was a gradual fall in weight with some recovery towards the end of the study. No significant weight changes were noted for cat 2.

Respiratory rate. The results of these observations are shown in Fig. 11.20.

Although the respiratory rate was always assessed before the animals were removed from their cages, it fluctuated greatly in both animals from week to week. Nevertheless, with cat 1, the trend was for a slower rate after thyroid ablation, possibly as a result of its being less nervous when approached. There were no obvious differences with cat 2. No abnormalities were noted on auscultation of the lungs at any stage during the study.

Heart rate. The results of these observations are shown in Fig. 11.21.

With both cats, the trend was for the heart rate to be slower after ablation, the rates usually falling below the commonly quoted

mean of 197 beats per minute (Tilley, 1985). Both animals showed actual, although marginal bradycardia (less than 160 beats per minute) after treatment, with the first occasion being as early as week 11 in cat 2.

Rectal temperature. The rectal temperatures of the two cats are illustrated in Fig. 11.22.

Both animals showed the same trends. From week 18, temperatures within the normal range were recorded on only two occasions from each animal.

Other systems. Neither cat showed signs of gastrointestinal abnormalities during the period of study.

Although both animals were ex-breeding queens, no oestrous behaviour was noted during the experimental period.

Haematological tests

Red blood cells. These data are presented in Fig. 11.23.

Although the RBC counts of both cats remained in the normal range throughout the experiment, they were consistently below the individual pre-treatment values from week 12 in both animals. No abnormalities of the red cells were detected.

Packed cell volume. These data are presented in Fig. 11.24.

The PCV of both cats also remained in the normal range for the duration of the study. However, from weeks 9 (cat 2) and 15 (cat 1), the values obtained were consistently below those found before treatment.

Haemoglobin concentration. These data are presented in Fig. 11.25.

Although the haemoglobin concentration of both animals remained in the normal range throughout the study, from weeks 9 (cat 2) and 16 (cat 1), the values were consistently lower than those obtained before treatment. Cat 1 appeared to be most severely affected but showed some increase in haemoglobin concentration towards the end of the experimental period.

Erythrocyte sedimentation rate. These data are presented in Fig. 11.26.

Prior to thyroid ablation, the ESR of each cat was low and relatively constant. After treatment with radioiodine, the ESRs of both animals fluctuated dramatically and were frequently above the upper limit of normal. These fluctuations became less in the later part of the study, although abnormal values were still recorded for both animals.

Reticulocyte counts. These data are presented in Fig. 11.27.

Prior to administration of radioiodine, the reticulocyte count was in the normal range in both animals. Contrary to what might have been expected, elevated counts were recorded on a small number of occasions after thyroid ablation.

Biochemical tests

Serum thyroxine concentrations. These data are presented in Fig. 11.28.

For both cats, the serum total T4 concentrations were consistently below the detection limits of the assay ($<1.3 \text{ nmol/l}$) from the time of first sampling after administration of Na^{131}I (week 9). From weeks 16 (cat 1) and 18 (cat 2), the per cent binding of tracer in the samples was greater than that in the RIA zero standard.

Serum triiodothyronine concentrations. These data are presented in Fig. 11.29.

For both cats, the serum total T3 concentrations were consistently below the detection limits of the assay (0.1 nmol/l), with the per cent binding of tracer in the samples being greater than that in the RIA zero standard, from the time of first sampling after administration of the radioiodine.

Cholesterol. These data are presented in Fig. 11.30.

The serum cholesterol concentrations of both cats were within the normal range both before and after thyroid ablation. There was no tendency for the serum cholesterol concentration to rise during monitoring of the hypothyroid state.

Creatinine kinase. These data are presented in Fig. 11.31.

Values above the normal reference range were seen both before and after thyroid ablation in both cats and no trends were obvious for this analyte.

Serum protein concentrations. These data are presented in Figs. 11.32 and 11.33 and Table 11.1.

Total serum protein. The total serum protein concentrations of both cats were found to increase after thyroid ablation, such that from weeks 12 (cat 2) and 22 (cat 1) they were consistently elevated.

Serum albumin. The serum albumin concentration of both cats tended to decrease after administration of radioiodine. With cat 1, between weeks 16 and 25, and cat 2, between weeks 16 and 38, the values were consistently below the reference range. The concentrations were never low enough to precipitate ascites or oedema.

Serum alpha-1-globulin. No trends were obvious with this protein fraction in either cat.

Serum alpha-2-globulin. No general trends were obvious with this protein fraction. In cat 1, the concentration decreased (although remained in the normal range) between weeks 14 and 22 but similar changes were not observed in cat 2.

Serum beta-globulin. No general trends were obvious with this protein fraction.

Serum gamma-globulin. In cats 2 and 1, the concentrations of gamma-globulin became elevated from weeks 11 and 12 respectively. Although there was some degree of fluctuation between samples, the general trend was for the concentrations to increase steadily with time, such that the final serum samples showed gamma-globulin concentrations of greater than twice the upper limit of the normal reference range.

Skin biopsy

The thickness of the viable epidermis varied depending on the site. Biopsies from the lateral thorax, lateral abdomen, xyphysternum and the dorsal mid-thorax (both cats) and the anterior carpus (cat 1) showed a single layer of nucleated epidermal cells. The epidermis of the inner and outer surfaces of the pinnae of both cats was acanthotic with up to six layers of nucleated cells (Fig. 11.34). In this site, melanin could be found throughout the epidermis. Hyperkeratosis (Fig. 11.34) was evident in all biopsies except those from the muzzle and the dorsal mid-thorax.

Increased dermal mucin was present in a number of sites. It was most obvious in the pinnae of both cats, in the deep dermis on both sides of the cartilage (Fig. 11.35). It was also seen in

connective tissue deep to cutaneous muscle in the muzzle and around the hair follicle units and in the frontal region and the lateral thorax (both cats), the dorsal mid-thorax (cat 1) and the tail-base (cat 2). Collagen fibres appeared normal in all sites with the exception of the pinnae where, in both cats, they were smaller, fragmented, less eosinophilic with haematoxylin and eosin, and showed reduced numbers of nuclei (Fig. 11.36). This change was most marked in the deep rather than the superficial dermis, especially in cat 1. No abnormalities of elastic fibres were detected.

In the dermis of the outer surface of the pinnae (both cats) and in the anterior carpus (cat 2), there was pigmentary incontinence with dermal melanophages.

Hair follicles contained hairs, even in sites which were macroscopically alopecic (Figs. 11.37 and 11.38). Follicular hyperkeratosis was absent but there was a light, mononuclear cell infiltrate around the hair follicle units. No abnormalities of arrector pilae muscles were identified.

No abnormalities of sebaceous glands were seen. In contrast, apocrine gland changes were noted in sections from all sites except the muzzle and the pinnae in one or both cats. These abnormalities ranged from a reduction in number (cat 1, dorsal mid-thorax; cat 2, lateral thorax, lateral abdomen), partial atrophy and shortening with a lining of flattened cells (cat 1, frontal region, dorsal lumbar area; cat 2, lateral abdomen), to their complete absence. In only one site (the anterior carpus), were apocrine glands normal in one animal and absent in the other (cats 1 and 2 respectively).

Electrocardiography

Post-ablation ECGs from the two cats are shown in Figs. 11.39 and 11.40. The data are quantified in Table 11.2.

For both cats, the majority of values were within the normal respective limits as published by Tilley (1985). P.G.G. Darke (personal communication) considered all the values to be within the normal respective ranges for cats.

11.7 DISCUSSION

Preparation of animals and induction of hypothyroidism

Hypothyroidism can be induced surgically, medically or with radioiodine.

Because of the frequency with which accessory thyroid tissue is found in dogs, surgical thyroidectomy is not universally successful in producing hypothyroidism (DiScala, Lippe and Segal, 1971). Accessory thyroid tissue is equally common in cats (see Section 3.4), and the outcome of surgical ablation is, therefore, likely to be similar. Indeed, bilateral surgical thyroidectomy for feline hyperthyroidism does not usually necessitate thyroid hormone replacement therapy (see Chapter 10), suggesting that accessory thyroid tissue quickly becomes functional after removal of the thyroid glands.

Goitrogens such as the thiouracils have been used in the cat to induce reported (although unconfirmed) hypothyroidism (Pascalov-Stoenescu and Sterescu, 1971) but mild to severe side-effects of such therapy occur frequently in cats (Peterson, 1981; Peterson et al., 1984). Consequently, the safest and most effective agent for the experimental induction of feline hyperthyroidism is radioiodine.

In the dog, doses of up to 3 mCi/kg ^{131}I have been used without causing total thyroid ablation (Goldberg and Chaikoff, 1952). Varying, although considerably smaller doses have been reported to result in hypothyroidism in cats, an apparently effective dose being 1 mCi/kg (see Section 3.12). Consequently this dose was used in the present study. However, a number of other procedures were employed to ensure maximal thyroidal uptake of the radioisotope.

The cats were fed on an iodine-deficient diet (fresh bovine heart) for five weeks prior to administration of the radioisotope, to deplete body stores of iodine. Bovine heart contains negligible amounts of iodine (Scott, Greaves and Scott, 1961). In addition, heart also has a very low calcium content and the same authors showed that the feline thyroid concentrated 2.5 times the quantity of ^{131}I when animals were fed on raw heart alone, compared with those fed raw heart supplemented with calcium gluconate at 2.5g/100g wet diet (Scott, Greaves and Scott, 1961).

Deionised water was supplied during this period and for three days after the administration of the radioiodine. In addition, animals were starved for three days before and three days after dosing. It was hoped that this would markedly reduce the urinary excretion of ^{131}I and thereby increase its thyroidal uptake. This effect was expected from the report by Riggs (1949) who noted a marked dependence of iodide clearance on chloride excretion in dogs, with prolonged retention of iodide by animals whose kidneys were conserving salt. Lippincott, Lewallen and Shellabarger (1957) confirmed this effect during their studies on canine thyroid ablation using ^{131}I . Starvation appeared to cause no untoward

effects in the two cats in this experiment. Indeed, Anderson and Lewis (1980) found that dogs undergoing total calorie restriction exhibited less hunger after the first few days than dogs with partial calorie restriction, and no significant clinical or biochemical changes were detected in periods of total starvation ranging from 24 to 42 days.

In addition, TSH was given 24 and four hours before administration of the Na^{131}I . TSH increases thyroidal trapping of iodide after several hours (Belshaw, 1983) and has been used to increase ^{131}I uptake during the production of experimental hypothyroidism in dogs (DiScala, Lippe and Segal, 1971).

Monitoring of the hypothyroid state

Confirmation by determination of serum thyroxine and triiodothyronine concentrations. Prior to administration of radioiodine, the mean total T4 and T3 concentrations of sera taken on four occasions from the cats in this study were 37.7 nmol/l and 0.31 nmol/l (cat 1) and 46.2 nmol/l and 0.56 nmol/l (cat 2). In some samples, T4 concentrations were above the normal range as determined in Chapter 7. The reasons for this are unclear. From the first sampling four weeks after the administration of radioiodine, the serum T4 and T3 concentrations of both animals were consistently below the detection limit of the respective assays, confirming them to be hypothyroid. The finding that the per cent binding of tracer in the samples was greater than that in the zero standard suggests that there was little, if any, thyroid hormone in these sera.

Physical parameters

General demeanour. Both cats in this study were lethargic between weeks 17 and 38 and slept for long periods of the day.

Hypothyroid humans show similar signs and may lapse into stupor or even coma (Weiner, 1978). The reasons for these changes are complex. Thyroid hormones appear to interact with the nervous system. Scheinberg, Stead, Brannon and Warren (1950), in a study of eight hypothyroid patients, found reductions in cerebral blood flow and oxygen and glucose consumption and increased cerebral vascular resistance, suggesting a correlation between disturbances in brain metabolism and mental status.

From week 40, both cats reverted to normal activity despite continued physical and biochemical evidence of severe hypothyroidism. The reasons for this are unclear as such improvements in untreated humans do not occur.

Appetite, weight and rectal temperature. Apart from a period of inappetence from weeks 14 to 27, the appetite of both animals remained unchanged during the period of study. The weight of cat 2 was unaltered, although cat 1 lost weight during the middle part of the study. The reasons for the latter observation are unclear.

The rectal temperatures of both animals were subnormal almost constantly from week 18.

In human hypothyroidism, there is a slowing of a vast number of energy-requiring reactions, which is reflected in a decreased rate of oxygen consumption per unit of body surface area and a decrease in the rate of heat production (Du Bois, 1936). The decreased rate of energy utilisation and heat production are shown clinically as a fall in basal metabolic rate, a decrease in body temperature and cold intolerance. There is mild weight gain, despite a reduction in appetite, in approximately half of hypothyroid humans

(Watanakuakorn, Hodges and Evans, 1965), although obesity is rare (Loeb, 1978). Obesity or weight loss, decreased or increased appetite and a low body temperature may be associated with canine hypothyroidism (Muller, Kirk and Scott, 1983).

Respiratory system. The respiratory rate of cat 1 tended to decrease after thyroid ablation although there was no obvious change with cat 2. No abnormalities were detected on auscultation at any stage with either animal. No other studies of respiratory function were carried out. In man, development of severe hypothyroidism does not give rise to consistent significant changes in lung volume, airway conductance or blood gas composition. In severe myxoedema, however, there is evidence of alveolar hypoventilation with hypercapnia and hypoxia that may be severe enough to induce coma (Turino, 1978).

Cardiovascular system. In both cats, the trend was for a slower heart rate in the hypothyroid compared with the euthyroid state. Bradycardia was occasionally seen in both animals.

Bradycardia is a common feature of human hypothyroidism. It appears to be due to the effect of lack of thyroid hormone on the electrophysiological properties of sinoatrial cells. Decreased sympathoadrenal stimulation may also be contributory (Skelton and Sonnenblick, 1978).

No significant alterations were seen on ECGs from either cat. In human hypothyroids, the ECG may also be normal, but characteristic though non-diagnostic changes (sinus bradycardia, flattening or inversion of the T wave and low P, QRS and T wave amplitudes), are usually seen (Douglas and Samuel, 1960). Both the direct myocardial effects of thyroid hormone deficiency and pericardial effusion are

important in the genesis of the ECG changes seen (Skelton and Sonnenblick, 1978). No tests were carried out to detect possible pericardial effusions in the present study.

Skin changes. Skin changes were seen in both cats in this study and are common in both human and canine hypothyroidism (Roberts and Rook, 1979; Belshaw and Rijnberk, 1980). Although the same types of changes occurred in both animals, they began earlier and were always more severe in cat 2. Variations in the dermatological manifestations of canine hypothyroidism have been reported to be enormous and frequent (Muller, Kirk and Scott, 1983).

The earliest change seen in both cats was diffuse thinning of hair over the trunk. Thyroid hormones have a number of effects on hair growth, initiating or advancing anagen and increasing growth rate (Amoroso and Ebling, 1966). Although coat changes do not occur universally in hypothyroid dogs, in many, hair follicles remain in telogen and there is failure of hair growth (Muller, Kirk and Scott, 1983). Thinning of hair first develops over areas such as the neck, the dorsal aspect of the nose, the pinnae, the trunk and the tail, where friction tends to dislodge telogen hairs (Martin and Capen, 1979), but may progress to generalised, bilaterally symmetrical alopecia. Widespread hair loss did not develop in the cats in this study and alopecia was confined to the pinnae, the carpi (both cats) and the tail-base (cat 1). Widespread alopecia did not occur in a congenitally hypothyroid kitten (Arnold et al., 1984) nor in other cats in which the thyroids were ablated using ^{131}I (Crawford, 1961). Hair loss is reported to occur in up to 49 per cent of hypothyroid humans (Saito, Hou and Kurebayaski, 1976). Typically, there is loss of the lateral third of the eyebrows and sometimes the

sexual hair and beard. Hairs in these areas grow only slowly (Lang, 1978) and it is interesting that, in the present study, the most severe hair loss was over the pinnae, an area where hair is short and growth is slow.

In human hypothyroid patients, there is a decrease in numbers of anagen hairs only in those patients with clinical hair loss (Smith, Weinstein and Burr, 1959). In the cats in the present study, widespread alopecia did not develop and hair repeatedly removed by clipping in multiple sites, regrew. Although plucked hairs were not examined to determine anagen: telogen ratios, these results suggest that no major change followed as a consequence of the hypothyroid state.

Both cats had dry and brittle coats. The hair of hypothyroid humans and dogs is also dull, coarse and brittle (Lang, 1978; Muller, Kirk and Scott, 1983).

Both cats developed abnormal keratinisation reflected as varying degrees of dry scale. Similar changes have been reported previously in experimental feline hypothyroidism (Randall and Parsons, 1970). Hypothyroidism in man and dogs results in abnormal keratinisation due to decreased protein synthesis, mitotic activity and oxygen consumption (Holt, Lazarus and Marks, 1976; Martin and Capen, 1979). In hypothyroid people, decreased activity of eccrine (merocrine) sweat glands and sebaceous glands also contributes to dry skin (Warin, 1973). Eccrine sweat glands are found only in the footpads of cats (Strickland and Calhoun, 1963) and effects of hypothyroidism on them could not, therefore, have contributed to the dry skin seen in the cats in the present study.

Hyperkeratosis and hyperpigmentation of the pinnae developed in both cats. Identical macroscopic changes were seen in a cat with clinical and biochemical signs of hypothyroidism subsequent to bilateral thyroidectomy for hyperthyroidism (see Chapter 10). Hyperpigmentation may occur in hypothyroid humans and dogs (Muller, Kirk and Scott, 1983).

In human hypothyroidism, myxoedema is most obvious over the face, producing a broad, flat nose (Freinkel and Freinkel, 1971) and hypertelorism (Adams and Reed, 1971). Both cats in the present study developed apparent shortening and broadening of the muzzle which may have been due to myxoedema. Similar changes were reported in a case of goitrous primary hypothyroidism in a kitten (Arnold et al., 1984).

A number of other skin changes occur in hypothyroid humans and dogs. The skin may be cold due to vasoconstriction. There may be poor wound healing, capillary fragility and easy bruising (Lang, 1978; Muller, Kirk and Scott, 1983). Lack of thyroid hormones may also affect resistance to infection in man (Lang, 1978) and pyoderma is a common complication of the condition in dogs (Muller, Kirk and Scott, 1983). Comedo formation is seen frequently in canine hypothyroidism (Scott, 1982a). None of these changes was seen in the cats in this study.

A number of histological abnormalities were seen in skin biopsies from these cats. Hyperkeratosis was identified in specimens from most sites. This finding must be carefully evaluated, as the processing and subsequent staining of sections of normal feline skin alter the structure of the stratum corneum from a compact to a loose formation (Baker, 1974a). In this study,

biopsies were processed in agar which prevents loss of keratin even if it is loose. In the present report, the term hyperkeratosis is used to describe a stratum corneum of thickness equal to or greater than a four cell thickness of the stratum spinosum.

The epidermis of these cats was one to six nucleated cells thick, depending on the site. Epidermal atrophy has been reported in some cases of canine hypothyroidism (Rojko, Hoover and Martin, 1978). The epidermis of normal feline hairy skin is two to three nucleated cells thick (Baker, 1974a) and in cats, the term epidermal atrophy can therefore only be ascribed to areas where there is a single layer of basal epidermal cells. This occurred over the dorsal mid-thorax, lateral thorax, xyphysternum and lateral abdomen (both cats) and the anterior carpus of cat 1.

Although Baker (1974a) stated that the stratum spinosum was not present in normal, feline, hairy skin, most other workers (e.g. Strickland and Calhoun, 1963; Scott, 1980) disagree, stating it to be one or two cells in thickness. Acanthosis (a stratum spinosum of more than three cells thick) was identified at the inner and outer pinnae surfaces of both cats. In these sites, melanin could be found throughout the epidermis. In cats, melanocytes are found in the germinal layer of only a few specialised, relatively hairless, skin areas: the prepuce, scrotum and teats (Strickland and Calhoun, 1963), the circumanal area, the pinnae and the umbilical skin of the foetus (Baker, 1974a). Epidermal melanosis may occur in hypothyroid humans and dogs but the pathomechanism is unclear (Muller, Kirk and Scott, 1983).

The major dermal abnormalities seen were increased dermal mucin and swollen, fragmented collagen fibres. Mucin is a mixture

of acid mucopolysaccharides, particularly hyaluronic acid and chondroitin sulphate. Hyaluronic acid has a high affinity for water and its deposition results in puffy, non-pitting skin (Freedburg, 1978b). Originally, it was felt that the increase in dermal mucopolysaccharides resulted from high circulating TSH concentrations (Dyrbye, Ahlquist and Wegelius, 1959). However, the same deposits are found in secondary hypothyroidism and it is unlikely that TSH is important in the process. Thyroid hormone deficiency probably accounts for mucin accumulation (Asboe-Hansen, 1966).

It is generally considered that the dermal mucin of both human and canine hypothyroidism is not readily visualised with the haematoxylin and eosin technique (Lang, 1978; Martin and Capen, 1979), and special stains such as Alcian blue or PAS must be used to demonstrate the material adequately (Milne, 1972). However, in this study, haematoxylin and eosin were equally as effective in demonstrating myxoedema as the more specialised stains.

In human hypothyroid patients, mucin is found predominantly in the papillary dermis. However, because of a lack of rete ridges and dermal papillae, there is no clear-cut division into a papillary and reticular dermis in cat skin and the terms superficial and deep dermis are preferred (Scott, 1980). Myxoedema was found around the hair follicle units and in the deep dermis, particularly in the skin of the muzzle and the pinnae.

Swollen, fragmented collagen fibres were seen in the skin of the pinnae of both cats. Such changes are a feature of some cases of human and canine hypothyroidism (Percival, Montgomery and Dodds, 1962; Martin and Capen, 1979). The physical compression of

collagen by mucin is thought to contribute to these changes (Martin and Capen, 1979). In experimentally-induced hypothyroidism in various animal species, it has been shown that the rates of collagen synthesis and degradation are decreased (Fink, Ferguson and Smiley, 1967; Kivirikko, Laitinen, Aer and Halme, 1967) and this may also contribute to the changes seen in the naturally-occurring condition.

Histologically, there was no obvious change in the anagen:telogen ratio of hair follicles from either cat except over the distal halves of the pinnae where telogen follicles predominated. In this area, there was, in addition, a light, mononuclear cell infiltrate. Appendagocentric inflammatory infiltrates have been described in canine hypothyroidism (Scott, 1982a).

Abnormalities of sebaceous glands were not identified in the present study. Decreased activity of sebaceous glands (Lang, 1978) and sebaceous gland atrophy (Scott, 1982a) are common in human and canine hypothyroidism respectively.

Changes in the appearance and number of apocrine sweat glands were seen in many of the biopsies. Although Langham and Schirmer (1968) observed cystic changes in, and Anderson (1979) reported atrophy of, apocrine glands in canine hypothyroidism, Scott (1982a) found no evidence of such changes in skin biopsies from 137 confirmed hypothyroid dogs.

Eccrine sweat glands are found only in the footpads of cats (Strickland and Calhoun, 1963) and biopsies from these areas were not examined in the present study. Specific cytological changes have been reported in the eccrine sweat glands of human hypothyroid patients (Means and Dobson, 1963).

A large number of other histological abnormalities have been reported in skin from hypothyroid dogs (Scott, 1982a) but were not identified in the present study.

Other systems. Abnormalities may be seen in a number of other systems in human hypothyroidism. Intestinal motility disturbances are the major and most frequent gastrointestinal effects of the disease, with patients complaining of distention, flatulence and constipation (Javitt, 1978). No evidence of gastrointestinal abnormalities was detected in the two cats under discussion.

Amenorrhoea is common in hypothyroid women (Hembree and Vande Wiele, 1978). Neither of the two experimental cats showed evidence of oestrous behaviour. Enlarged mammary glands and galactorrhoea may also be seen in human and canine primary hypothyroidism (Hembree and Vande Wiele, 1978; Chastain and Schmidt, 1980). It is thought that these result from increased TRH secretion which stimulates increased prolactin release. No such changes were seen in the two experimental cats.

Haematological tests. The RBC counts, PCVs and haemoglobin concentrations of both cats were consistently lower after thyroid ablation than before treatment, although no subnormal values were recorded. The reticulocyte counts remained in the normal range apart from on a small number of occasions. As the lower limit of the reticulocyte range in healthy cats is zero in the author's laboratory, it is not possible to draw any conclusions about the effect of thyroid ablation on this parameter. Certainly, there were no major changes and elevated counts were actually recorded on a number of occasions.

In human medicine, approximately one third of all patients with hypothyroidism have anaemia which has a primary and sometimes a secondary component (Tudhope and Wilson, 1960). The primary component, which is dominant in fifty per cent of patients, results in a physiological, hypoplastic (normocytic, normochromic) anaemia as an adaptive response to decreased tissue oxygen requirements. It is responsive to thyroid hormone alone. Superimposed on this, there may be a secondary nutritional anaemia due to deficiency of iron, vitamin B12 or folate, or any combination of these. These deficiencies may arise from poor appetite, intestinal malabsorption or as a result of autoimmune disease (pernicious anaemia). If iron deficiency anaemia (responsive to iron alone) is dominant, the red cells are hypochromic and microcytic. If vitamin B12 (responsive to vitamin B12 alone) or folate deficiency is dominant, the red cells are macroovalocytic.

In the present study, the red cells appeared normal and their reduced numbers appeared to correspond to the hypoplastic situation in man. Cline and Berlin (1963), in investigations on erythropoiesis and red cell survival in experimentally-induced hypothyroidism in dogs, reported similar findings. Parenteral iron or parenteral vitamin B12 administration had no effect on the haematocrit or reticulocyte count of these dogs. Leptocytes, reflecting increased membrane cholesterol loading, may be seen in canine hypothyroidism (Feldman and Feldman, 1977) but were not seen in blood samples from cats in this study (see subsequently).

The ESR of both animals was frequently elevated after thyroid ablation. Elevations of ESR are common in human myxoedema (McAlpine, 1955) and in canine hypothyroidism (Bush, 1978).

Elevations of the ESR may result from concurrent increases in plasma concentrations of fibrinogen and, to a lesser extent, alpha and gamma-globulins (Zilva and Pannall, 1975). The plasma gamma-globulin concentrations of both these cats increased progressively after treatment with radioiodine. While this may have been contributory to the raised ESR, it could not have been the sole cause as higher ESRs were seen in the earlier, and gamma-globulin concentrations in the later, part of the study. ESR also increases with tissue injury and haemodilution (Schappes, 1937), and the high rates seen in the earlier part of the study may have resulted from thyroidal damage by the radioiodine and the fall in RBC numbers which was most severe at this time.

Non-specific biochemical tests

Cholesterol. There was no trend for increases in the serum cholesterol concentration after thyroid ablation in these animals. In man, hypercholesterolaemia has been seen in up to 80 per cent of hypothyroid patients (Watanakuakorn, Hodges and Evans, 1965). Cholesterol biosynthesis is decreased in such patients (Loeb, 1978) but its degradation is decreased to a greater extent (Koppers and Palumbo, 1972). There is a three-fold increase in the plasma concentration of low-density, cholesterol-carrying apolipoproteins, reflecting decreased cholesterol catabolism and turnover (Walton, Scott, Dykes and Davies, 1965). These combined effects appear to account for the hypercholesterolaemia in human hypothyroidism. Approximately 33 per cent of hypothyroid dogs show an elevation of serum cholesterol concentrations (Muller, Kirk and Scott, 1983).

Experimental feline hypothyroidism does not appear to be accompanied by significant increases in serum cholesterol. This may

account for the absence of leptocytes on haematological examination (already described).

Creatinine kinase. Values for this analyte were above the normal reference range in both animals before and after thyroid ablation and no trends are obvious from these data.

In man, hypothyroidism is associated with a wide spectrum of muscle symptoms and signs (McKeran, Slavin, Ward, Paul and Mair, 1980). The muscle enzyme of CK is elevated in up to 80 per cent of human hypothyroids (Fleisher, McConaley and Pankow, 1965).

Serum proteins. After thyroid ablation, there were a number of alterations in serum protein parameters. Albumin concentrations tended to fall. In human hypothyroidism, similar findings have been described (Lewis and McCullagh, 1944; Skillern, Crile, McCullagh, Hazard, Lewis and Brown, 1956), although other workers have reported that, despite a reduction in the synthesis of albumin, there is a proportionately greater decrease in its degradation resulting in a rise in the total miscible albumin pool (Schwartz, 1955; Lewallen, Rall and Berman, 1959).

No trends were obvious for the alpha or beta-globulin fractions. The increased serum cholesterol of some human hypothyroid patients is reflected on electrophoresis by an elevation of the beta-lipoproteins, either alone or in association with elevations in pre-beta-lipoproteins (Jones, Cohen and Corbus, 1955). Similarly, in lipaemic hypothyroid dogs, there may be intense electrophoretic bands at the origin, the beta-1-lipoprotein and the alpha-2-lipoprotein positions (Rogers, 1977). Specific lipoprotein electrophoresis was not carried out on samples from the cats under discussion. However, large increases in serum lipids may be obvious

on serum protein electrophoresis. No such changes were observed in the two experimental cats, the serum samples were not lipaemic, nor were there increases in serum cholesterol concentrations after thyroid ablation.

In both cats, the serum gamma-globulin concentrations increased markedly and progressively after thyroid ablation. The increases in this serum protein fraction were responsible for the increases in the total serum protein concentrations.

Levin and Leathem (1942) showed that surgical thyroidectomy in rats increased the serum gamma-globulin concentration and that treatment with T4 prevented this change. Hypergammaglobulinaemia has been known, for a considerable period of time, to occur in Hashimoto's disease in man (Luxton and Cooke, 1956), and Skillern et al. (1956) felt that this was due entirely to deficiency of thyroid hormones. However, Roitt et al. (1956) showed that, at a time when Hashimoto's disease was still being treated by thyroidectomy, the gamma-globulin concentrations returned to normal after surgery. Lindsay, Dailey and Jones (1954) reported that thyroidal histological patterns indistinguishable from those in Hashimoto's disease were observed after therapeutic doses of ^{131}I in humans. As thyroglobulin is released after radioiodine therapy, Doniach and Vaughan Hudson (1957) suggested that the immune response in Hashimoto's disease represented an immune response to antigen released by the thyroid. The possible role of thyroid autoantibodies as a source of the hypergammaglobulinaemia in the two cats under discussion was investigated subsequently and the results are reported in Chapter 14.

11.8 ATTEMPTS TO MEASURE SERUM THYROID STIMULATING

HORMONE CONCENTRATIONS IN CATS

Introduction

The value of measurements of plasma basal TSH in the diagnosis of human hypothyroidism has been discussed in Section 3.7. TSH is a species specific polypeptide. Attempts to measure canine TSH using different human TSH RIA kits have been variably reported as unsuccessful (Chastain, 1978) or reliable (Nachreiner, 1981a).

Feline TSH has not been purified and there are no reports of attempts to determine its serum concentration using heterologous RIAs. This section describes attempts to assay feline serum TSH using human and canine TSH assay procedures.

Methods

Radioimmunoassay for human thyroid stimulating

hormone. Sera were assayed courtesy of Dr. J. Seth, in a double antibody RIA using a sheep anti-human TSH (obtained from SAPU) as first antibody and ^{125}I -human TSH as radiolabel. The assay was standardised against the international reference preparation (IRP) of human TSH (80/558). Between-assay precision was approximately eight per cent cv at concentrations of 2mu/l or above, and the sensitivity was 0.9 mu/l.

Sera from two healthy pet cats, two experimental cats before and 17 weeks after thyroid ablation using radioiodine, and two animals with FEA (one before and 43 weeks after beginning treatment with 100 ug T3 daily) were examined.

Radioimmunoassay for canine thyroid stimulating

hormone. The kit used was a double antibody RIA employing rabbit anti-canine TSH as first antibody and ^{125}I -canine TSH as

radiolabel, (cTSH, obtainable from Associate Biolabs Ltd., The Novo Centre, 9-11 London Lane, London, England). Within and between-assay precision were approximately seven and eight per cent cv respectively at concentrations of 5.2 ng/ml or above. The sensitivity of the assay was not stated. All samples were tested in duplicate.

Sera from six healthy pet cats, two experimental cats before and on three occasions after thyroid ablation using radioiodine, and eight cats with FEA prior to and two during T3 therapy were tested. In addition, canine sera from two cases of primary and two cases of secondary hypothyroidism, confirmed by the measurement of T4 before and after TSH stimulation testing, and in one case, at post-mortem examination (139943) were included to act as controls.

Radioimmunoassay for thyroxine and triiodothyronine.

Feline sera were assayed for T4 and T3 as described in Chapter 6. T4 and T3 concentrations in canine sera were determined by a commercial laboratory using an RIA method optimised and validated for dogs (Serono Laboratories (UK) Ltd., 2 Tewin Court, Welwyn Garden City, Hertfordshire, England.)

For all techniques, sera were stored at -40°C prior to testing.

Results

Radioimmunoassay for human thyroid stimulating hormone. These results are summarised in Table 11.3.

All TSH concentrations were at, or marginally above, the detection limit of the assay, even in the thyroid ablated animals in which high concentrations would have been anticipated. The slight differences in TSH concentrations that were seen in these cats pre and post-ablation and in the treated FEA cat, were not judged to be of any significance.

Radioimmunoassay for canine thyroid stimulating hormone. These results are summarised in Table 11.4.

Both dogs with primary hypothyroidism showed elevated TSH concentrations whereas those with secondary hypothyroidism showed concentrations below the assay detection limit, suggesting probable satisfactory performance of the technique. Both healthy dogs also showed TSH values which were below the assay detection limit.

In the experimental cats, TSH concentrations were below the detection limit of the assay, both before and after thyroid ablation. Measurable concentrations were recorded in the FEA cats, but these did not differ significantly from healthy animals when compared by the Mann-Whitney U-test. Paradoxically, an apparent increase in TSH concentrations was recorded in two FEA cats treated with T3.

Discussion

In the absence of an established assay for feline TSH, the purpose of these experiments was to determine whether there might be sufficient cross-reactivity between feline TSH and human or canine TSH, to allow the use of these heterologous assays in the assessment of feline thyroid function. The failure of both assays to detect raised concentrations in the thyroid ablated cats was taken as clear evidence that no significant degree of cross-reactivity existed. This is in agreement with current concepts on the species specificity of TSH (Ferguson, 1984).

Although slight differences in apparent TSH concentrations were seen in the thyroid ablated cats in the human assay, and measurable concentrations were recorded in the FEA animals in the canine assay, these probably reflected non-specific responses in the RIAs. Such

responses may arise owing to the different serum matrix found in cat serum and the sera for which the assays are designed.

12. THYROID FUNCTION IN FELINE ENDOCRINE ALOPECIA

12.1 INTRODUCTION

The aetiology of FEA is unknown (see Section 3.13). While Martin and Capen (1975) have equated FEA with hypothyroidism, most other authors have stated that affected cats are not hypothyroid (Baker, 1974a; Scott, 1975b; Doering, 1976; Scott, 1976; Kirk, 1980; Scott, 1980, 1981). However, only Scott (1975b) has presented data to support this view.

Many cases of FEA respond to treatment with thyroid hormones (see Section 3.13). The present study was carried out to determine whether any cases of FEA may result from hypothyroidism.

12.2 METHODS

The case material comprised 51 consecutive cats referred with a tentative diagnosis of FEA. After a detailed history and physical examination, a number of laboratory tests were carried out.

Examinations for dermatophytes

All cats were screened for evidence of fluorescence with an ultra-violet (Wood's) light. The lamp used contained two ultra-violet sources and a magnifying lens and was obtained from The Burton Manufacturing Company, Santa Monica, USA.

Samples for microscopic examination for fungal elements were obtained by superficial dry skin scraping and by epilating hairs in affected areas by means of epilation forceps. Material was examined after warming in ten per cent potassium hydroxide solution. Fungal

culture was performed by aerobic incubation of small amounts of hair and skin scales on Sabouraud's glucose agar containing 0.4mg/ml chloramphenicol and 0.5mg/ml actidione (cyclohexamide), and nutrient agar (obtainable from Oxoid Ltd.), containing the same concentration of the above antibacterial and antifungal agents.

Examinations for ectoparasites

After close clipping of the hair with electric clippers, and moistening the skin with five per cent potassium hydroxide solution, deep scrapings producing capillary ooze were taken for examination for ectoparasites. The material was warmed with ten per cent potassium hydroxide and subsequently aliquoted into small amounts prior to microscopic examination.

Examinations for endoparasites

Faecal samples for examination for endoparasite eggs were obtained by manual removal per rectum or, where this was impossible, were collected by the owners. Subsequently, 2g faeces were macerated through a small sieve into an evaporating dish using 30ml saturated sodium chloride solution. The residue in the sieve was then washed with a further 30ml of the saturated saline. Two chambers of a McMaster counting slide were filled with the faecal suspension and examined microscopically.

Haematological and biochemical examinations

Blood samples were collected from conscious animals by jugular venepuncture after a minimum of 12 hours' starvation. Potassium EDTA tubes were used for samples for routine haematological examination and plain tubes for samples for biochemistry.

T4 and T3 concentrations were determined as described in Chapter 6 and haematological parameters and cholesterol concentrations as in Chapters 10 and 11.

Treatment

All cases were initially treated for fleas, at weekly intervals for six weeks, with a combination of topical dichlorvos (0.2 per cent w/w) and fenitrothion (0.8 per cent w/w) (Nuvan Top, Ciba-Geigy), together with routine vacuum-cleaning of the environment. Animals which had responded to this treatment after six weeks were diagnosed as flea infestation or probable flea infestation (see later) and blood sampling was repeated as described above. A definitive diagnosis of FEA was based on the history, physical examination, absence of ectoparasites macro or microscopically, negative ultra-violet light examination and failure to demonstrate dermatophytes microscopically or on culture, and no response to flea-control measures. In these cases, blood sampling was repeated 12 weeks after beginning T3 therapy and was carried out three hours after administering T3.

All cases of FEA were treated with liothyronine sodium (L-T3, Tertroxin, Glaxovet) at an initial dose of 20ug/cat twice daily, increasing by 10ug twice daily every third day, to a maximum of 50ug twice daily. Cats were reinspected three, eight and 12 weeks after beginning T3 treatment and intermittently thereafter, and the response subsequently classified as total (hair regrowth complete), partial (incomplete regrowth of hair) or no response (no change in the condition). Treatment of some cases in the last two groups was by:

- (i) L-thyroxine sodium (L-T4, Eltroxin, Glaxo) at an oral dosage of 50ug twice daily and subsequently 100ug twice daily.

- (ii) depot injections of a preparation containing a combination of four testosterone esters (Durateston, Intervet Laboratories). Each intramuscular injection consisted of testosterone propionate (3mg), testosterone phenylpropionate (6mg), testosterone isocaproate (6mg) and testosterone decanoate (10mg), administered initially at monthly intervals.
- (iii) megestrol acetate (Ovarid, Glaxovet) at an initial oral dosage of 5mg every third day, reducing after three weeks to 2.5mg every third day and subsequently to the lowest possible maintenance dosage (approximately 2.5mg once or twice weekly).

12.3 RESULTS

Wood's light examination, direct microscopy and culture for dermatophytes, examination of skin scrapings for ectoparasites and faecal examinations for endoparasite eggs were negative in all 51 cats.

Feline endocrine alopecia

Feline endocrine alopecia was diagnosed in 26 cats.

Historical data. Historical data for the FEA cats are presented in Table 12.1.

Twenty-four of the animals were domestic short-haired cats with two cross-breeds. There were ten neutered males (38.5 per cent), 15 neutered females (57.8 per cent) and one entire male (3.7 per cent). The age at onset ranged from one to 11 years with a mean of 4.5 years. Of the 23 neutered animals where the age of surgery

was accurately known, only one had been neutered above nine months of age. The latent period between neutering and the onset of FEA ranged from six months to 10.5 years with a mean of 4.1 years.

The duration of physical signs before referral ranged from two weeks to seven years.

No significant variation of onset of alopecia between the months was identified using the Chi-Square test.

Seven animals (26.9 per cent) showed symptoms of involvement of other systems in addition to the skin. These were lethargy (four cases), vomiting of hair (three), and obesity and inappetence (one each).

Seventeen of the cats had received some form of treatment before being referred. In 14 of these, there had been no improvement of the condition. Three cases had responded to a number of drugs but had relapsed at varying intervals after the cessation of treatment.

Physical and laboratory findings. The physical and laboratory findings for the 26 cats before and after treatment with T3 are presented in Table 12.2.

Pre-treatment data

Dermatological changes. The hair changes seen were as described in the literature and reviewed in Section 3.13. Generally, there was diffuse thinning in affected areas, rather than complete alopecia, although this varied considerably. The remaining primary hairs could be easily epilated. In some cases, secondary hairs could also be easily removed but, in others, epilation could only be achieved with difficulty.

The distribution of alopecia was very variable. Of the 26 cases, the ventral abdomen and medial hind legs as far as the stifles were affected in 25 (96.2 per cent), the lateral hind legs in 15 (57.7 per cent), the caudal hind legs in 13 (50 per cent), the fore-legs from elbow to carpus in 12 (46.2 per cent), the anogenital region and the lateral abdominal area in five each (19.2 per cent), the ventral thorax in three (11.5 per cent) and the axillae and lateral thorax in two each (7.7 per cent). Examples of alopecia distribution are shown in Figs. 12.1 to 12.8. In general, animals in which the problem was long-standing tended to be more severely affected but this was by no means universal, e.g. case 134590 had had lesions confined to the ventral abdomen and medial thighs for four years.

Heart rate. Data on heart rate were available for 12 cases. Six (50 per cent) had values below the normally accepted reference range. No animal had an elevated heart rate.

Rectal temperature. Data on rectal temperature were available for 13 cases. Three (23.1 per cent) had values below and one (7.7 per cent) above the normally accepted reference range.

Thyroid hormones. Serum thyroxine and triiodothyronine concentrations were measured in all cases. For T₄, one (3.9 per cent) was below and three (11.5 per cent) above the reference range. T₃ concentrations of all cases were within the reference range.

Cholesterol. Serum cholesterol concentrations were measured in 22 cases. Four (18.2 per cent) of these had values above the reference range. No animal had a cholesterol concentration below the reference range.

Red blood cell count. Data on RBC count were available for 17 cases. Sixteen animals (94.1 per cent) had values within the reference range. One animal had an RBC count which was marginally above the normal range.

Packed cell volume. Data on PCV were available for 17 cases. No values were below the normal range but three (17.7 per cent) were above the upper limit of the reference range.

Erythrocyte sedimentation rate. Data on ESR were available for 14 cases. Two animals (14.3 per cent) had values above the upper limit of the normal range.

Reticulocytes. Data on reticulocyte count were available for 15 cases. One animal (6.7 per cent) had a value above the reference range prior to treatment, while no sub-normal values were recorded.

Eosinophil count. Data on the eosinophil count were available for 17 cases. Only one value (5.9 per cent) was above the upper limit of the reference range.

Post-treatment data. Retrospectively, the 26 FEA cases could be divided into three groups according to their response to T3 therapy.

Historical data for these three groups are compared in Table 12.3 and show no obvious difference in the age at onset between them. Both the pedigree cats were T3 totally responsive. While there were equal numbers of neutered males and neutered females in the T3 totally responsive group, neutered females accounted for 75 per cent and 100 per cent of the partially and T3 non-responsive groups respectively.

Liothyronine totally responsive group. Data on treatment of these 19 cases are presented in Table 12.4.

In 13 cats, hair growth was obvious at the first reinspection after beginning T3 therapy (three weeks), although it was delayed as long as ten weeks in one cat. Complete regrowth of hair over affected areas had occurred by 22 weeks in all but one case. In black cats, the first signs of regrowth were multiple, discreet islands of black hair in affected areas. Examples of two cases in this group before and during T3 therapy are shown in Figs. 12.9 to 12.12.

Other symptoms associated with FEA resolved in three cats, and one other owner (of cat 131369) reported that the appetite and activity of her cat had improved although she had not initially reported these as being abnormal.

A Grade II (of V) soft, systolic, cardiac murmur with a split second sound developed in cat 134278 after three weeks of T3 therapy. The heart rate was 188/minute. No evidence of cardiac enlargement was seen radiographically but the ECG revealed evidence of left anterior fascicular block and ventricular premature beats (Fig. 12.13a). The dose of T3 was reduced to 40ug daily but the abnormalities were still present one week later. T3 therapy was stopped. On re-examination two weeks later, the murmur was absent at rest although a grade III systolic murmur could be heard when the heart rate increased due to excitement. On electrocardiography, there was no left anterior fascicular block although occasional ventricular ectopic beats persisted. Treatment was changed to monthly injections of depot testosterone. Three months later, hair was growing well and no cardiac abnormalities could be detected. An

ECG taken at this time is shown in Fig. 12.13(b). The cat was subsequently maintained with depot testosterone injections as required.

In the first three animals treated long-term with T3, the dose was reduced every four weeks by 20ug daily. In two, hair loss became evident at a dose of 80ug daily and in one at 40ug daily. The condition resolved once the T3 was increased to 100ug daily. In all but three subsequent cases, the lowest effective maintenance dose of T3 was determined. In three animals, T3 was reduced as described and eventually discontinued without recurrence of alopecia. Two animals (130196 and 131365) did not require subsequent treatment. One (108276) was treated at a dose of 100ug T3 daily when relapses occurred.

Liothyronine partially responsive group. Data on the treatment of these four cases are presented in Table 12.5.

In two animals the response was acceptable to the owners and the cats were maintained on 100ug T3 daily. Response to other treatments in two was complete.

Liothyronine non-responsive group. Data on the treatment of these three cases are presented in Table 12.6.

One cat also failed to respond to T4 and was lost to follow-up. Another was euthanased at the owner's request. The third animal failed to respond to T4 and treatment was discontinued for eight weeks. A thyroid biopsy was then carried out and the sections processed and stained as described in Chapter 10. No abnormality was noted. The animal subsequently responded completely to depot testosterone.

Comparison of the pre-treatment physical and laboratory findings according to response to liothyronine sodium. There were no obvious differences between the cutaneous lesions in the three groups of FEA cats. Other clinical and the laboratory data were compared using the Kruskal-Wallis test for group differences. The results of these comparisons are presented in Table 12.7.

Only the T4 concentration was significantly different between the groups, the inference from the test being that the more T3 responsive an FEA cat is, the lower is its basal T4 concentration.

Comparison of pre and post-treatment clinical and laboratory data for the liothyronine totally responsive group. Clinical and laboratory values for this group were compared before and 12 weeks after beginning T3 treatment, by means of Student's paired t test. The results are presented in Table 12.8.

The serum T4 and cholesterol concentrations were significantly lower and the serum T3 concentrations significantly higher 12 weeks after, compared with before, beginning T3 therapy. No other parameters studied were significantly altered.

Comparison of pre and post-treatment physical and laboratory findings for the liothyronine partially and non-responsive groups considered together. Because of the small numbers in each, these two groups were considered together. Data were compared before and 12 weeks after beginning T3 treatment, by means of Student's paired t test. The results are presented in Table 12.8.

The serum T4 concentration was significantly lower during T3 treatment. No other parameter was significantly altered.

Cases resembling feline endocrine alopecia

An alternative diagnosis to FEA was made in 24 of the 51 referred cases. One animal was lost to follow-up and was undiagnosed.

Historical data. Historical data of the 25 cases are presented in Table 12.9.

All were domestic short-haired cats. There was one entire male (4 per cent), three entire females (12 per cent), eight neutered males (32 per cent) and 13 neutered females (52 per cent). The age at onset ranged from less than one year to 12 years with a mean of 5.1 years. The duration of physical signs before referral ranged from one week to four years.

No significant variation of onset of alopecia between the months was identified using the Chi-Square test. In eight cases, pruritus had never been a feature of the problem, 11 had been pruritic (usually manifested by licking) from the onset and in five others, pruritus had been variable.

Fifteen animals had received some form of treatment before referral. In four of these, there had been no change in the condition. In 11, there had been temporary improvement with relapse on cessation of drug administration.

Physical and laboratory findings. Physical and laboratory data for the 25 cats at the first consultation are presented in Table 12.10.

Dermatological changes. In 19 animals, the distribution of the coat changes was very similar to FEA. In six cases, additional areas to FEA sites were affected. The per cent alopecia distribution of the group as a whole, and also of those animals in

which lesions were confined to FEA sites, are compared to the FEA cases in Table 12.11. This shows the very similar distribution of lesions in all three groups.

In 14 cases (56 per cent), hairs appeared to have been epilated rather than fractured. Fifteen cases (60 per cent) showed hair loss unaccompanied by skin changes.

Haematological and biochemical changes. Because a number of conditions were represented in this group, the haematological and biochemical changes within it were not compared.

The most pronounced abnormal laboratory finding was the number of blood eosinophils. Eleven animals showed an elevated (greater than $1.5 \times 10^9/l$) and three a high normal (greater than $1.1 \times 10^9/l$) total eosinophil count.

Diagnosis, treatment and outcome of the non-FEA group. These data are presented in Table 12.12.

Fleas or their faeces were found on nine animals. Topical treatment with a spray combination of dichlorvos and fenitrothion at weekly intervals for six weeks, together with vacuum-cleaning of the environment, produced remission of signs in all cases. One of these animals had kittened six weeks before treatment was begun and was diagnosed as having concurrent telogen defluxion.

In ten animals in which there was no evidence of fleas or their faeces on physical examination, flea treatment as described above resulted in remission of signs. An example of such a case is shown in Figs. 12.14(a) and 12.14(b). Five of these had elevated (greater than $1.5 \times 10^9/l$) and three high normal (greater than $1.1 \times 10^9/l$) total eosinophil counts on first presentation. These were diagnosed as probable flea infestation, and flea control

treatment as described above was carried out at weekly intervals. One cat had miliary dermatitis with eosinophilia. This was diagnosed as probable flea infestation but was lost to follow-up.

The diagnoses, treatment and outcome of the remaining cases are shown in Table 12.12.

Comparison of blood eosinophil counts before and six weeks after beginning treatment. In 14 cases, blood eosinophil counts were available before and six weeks after beginning treatment. In all cases, the post-treatment value was lower than the pre-treatment value. However, when these were compared using Student's paired t test, the results just failed to be statistically significant ($t = 1.91$; $P > 0.05$).

Comparison of historical, physical and laboratory parameters of the feline endocrine alopecia and non-feline endocrine alopecia groups

Table 12.13 compares the data for the month of onset of alopecia in the two groups.

Using the Chi-Square test, while there was no significant variation in frequency of onset of alopecia between the months for either group alone, such a variation was identified when the groups were considered together ($P < 0.05$). The onset of symptoms was greatest in the months March to May and October to December inclusive. Of the 43 cases in which the month of onset was known accurately, 35 (81.4 per cent) began during these six months.

In an attempt to identify an effective test for differentiating between FEA and clinically similar conditions, the clinical and laboratory data of the two groups were compared by the Wilcoxon Rank Sum test. The results are presented in Table 12.14 and show

significant differences between the two groups for the serum cholesterol concentration, the red blood cell count, the packed cell volume and a highly significant difference for the total eosinophil count.

Table 12.15 compares the eosinophil counts of the FEA animals and those clinically resembling FEA. The range in the FEA cats approximates to, and in the non-FEA cats is much greater than, the normal range. The median of the eosinophil count of cats with conditions resembling FEA is more than twice that of the FEA animals. The predictive values of the total eosinophil count in differentiating FEA from clinically similar conditions is shown in Table 12.16.

12.4 DISCUSSION

Although a number of workers have stated that FEA is not a manifestation of hypothyroidism, only one has presented supportive data (Scott, 1975b). However, the condition is frequently responsive to treatment with thyroid hormones (see Section 3.13). Accordingly, the aim of the present study was to determine whether, in some cases, FEA may result from thyroid hypofunction.

The parameters monitored were those used to assess thyroid function in the experimental study in Chapter 11. The clinical and laboratory changes occurring in hypothyroidism and the reasons for these changes were discussed in detail in that chapter and will not be considered further here. Because demodicosis (Scott, 1984) and dermatophytosis (Thoday, 1986) may present with similar lesions to FEA, screening tests to eliminate these conditions were carried out

on all cases at first presentation. They were negative in all 51 cats in this study. Preliminary observations suggested that the total eosinophil count might be useful in differentiating FEA from clinically similar conditions and it was therefore monitored in all cases. Because endoparasites may cause a circulating eosinophilia (Schalm, Jain and Carroll, 1975a), faecal samples from all animals were examined but parasite eggs were not identified in any.

Fleas are the most important cause of skin disease in cats (Thoday, 1985) and the clinical signs of infestation vary between individuals (Thoday, 1981). All cats were therefore treated with a parasiticide, and their environment by vacuum-cleaning, to exclude flea infestation as a cause of the presenting signs. Because there is no definitive test for FEA, this diagnosis had to be made on the history, results of physical examination, negative laboratory results for diseases with similar presenting signs and failure to respond to flea control measures.

Historical data in feline endocrine alopecia. FEA was not diagnosed in a pedigree cat in this study although two cross-bred cats were affected. Although Conroy (1964) stated that the condition was more common in the Siamese breed, and Kirk (1980), in a review of 34 cases, reported one further case in a Siamese cat, there are no other reports of the condition in pure-bred animals, although it has been described previously in cross-breeds (Scott, 1975b; Thoday, 1985).

Only one entire cat was affected in this series. Most other authors agree that the condition is more common in neutered individuals (reviewed in Section 3.13), although Kirk (1980) reported a relatively even distribution between entire and neutered

animals. As suggested by Thoday (1986), because the majority of mature cats seen at veterinary clinics are neutered, this predisposition may be apparent rather than real.

In the present study, 58 per cent of cases were neutered females and 39 per cent neutered males. No author has reported an apparent female predisposition for the disease. Indeed, Scott (1975b), in a series of 20 cases, found that 90 per cent were neutered males.

The age at onset of the condition in this series ranged from one to 11 years with a mean of 4.5 years. This is in good agreement with most other reports, although FEA is clearly not a disease of old cats as reported by Thornton (1963), Anon. (1966), Daykin (1971) and Joshua (1979).

In the current series, all but one animal had been neutered before nine months of age. Premature neutering has been suggested as a cause of the disease (Scott, 1981). However, feline castration and ovariectomy is routinely carried out at approximately six months of age in the UK. Kral (1959) and Kral and Schwartzman (1964) suggested that affected animals might produce insufficient androgens and/or oestrogens from their adrenal cortices, but no data were presented.

In the present series, there was a latent period between neutering and the onset of FEA, of between six months and 10.5 years, with a mean of 4.1 years. These data are in close agreement with those of Scott (1975b) who reported a range of one to nine years, with a mean of five years, in a series of 20 animals.

No significant variation in the frequency of month of onset of alopecia in the FEA group was identified in the present series.

However, the peak seasonal incidence was between November and May. This agrees with the findings of Kirk (1980) who found a "rough seasonal incidence" of the onset of FEA, although he did not analyse his data statistically. Although his cases were presented throughout the year, except for a higher concentration in November, the largest number of cases was seen between January and June, coinciding with the peak cat breeding season. These findings might have been expected from the studies of Baker (1974a) in Eire, who reported that in entire and neutered housed and unconfined cats, the hair roots showed minimum activity from December to May. Ryder (1976) in Australia, found that peak follicle inactivity was in August, corresponding closely to the results of Baker (1974a) in the northern hemisphere.

Seventeen animals had received a wide variety of drugs prior to referral. Most of the agents used have been recommended in the literature for the treatment of FEA (see Section 3.13). However, response in all but three cases had been unsatisfactory. In many cases, this may have been a dose-related problem because dosages were not specified by most referring veterinary surgeons. However, the failure rate with the progestogen megestrol acetate, a commonly recommended therapy for FEA, was very high.

Physical and laboratory findings in feline endocrine

alopecia. The distribution of hair loss in this series was in general agreement with literature reports of FEA. However, while the majority of authors have reported that the condition begins over the anogenital region, this was not the case in the present study where this area was affected in only 19 per cent of cases. Figures for the distribution of FEA have not been published previously.

Three animals were reported to vomit hairballs as a result of grooming in FEA. This is in agreement with the observations of Kunkle (1984).

While three animals were lethargic and one obese in association with the condition, systemic signs suggestive of hypothyroidism were absent in the remainder.

The pre-treatment laboratory data did not suggest hypothyroidism as the cause of FEA. The serum T4 concentration was below the reference range in only one animal. The T3 concentration was normal in all animals studied. No trends can be deduced from the non-specific tests of thyroid function.

Treatment of feline endocrine alopecia. While T4 is currently the therapy of choice for the treatment of naturally-occurring hypothyroidism in man (Toft, Campbell and Seth, 1981), dogs (Rosychuk, 1982) and iatrogenic hypothyroidism in cats (Holzworth et al., 1980), all cases of FEA in this series were treated with T3 because in a preliminary study, the clinical impression gained was that the response to T3 was better than to T4. An increasing dosage regime was used to prevent possible cardiac or thyrotoxic problems being brought about by initial slow metabolism of the hormonal supplement or rapid changes in cellular metabolism. Such regimes have been used in human medicine (Rosychuk, 1982). Despite this, one cat developed cardiac signs compatible with iatrogenic thyrotoxicosis during treatment with T3. These signs resolved completely when T3 was withdrawn.

A maximum total daily dose of 100ug T3 per cat was used. This is higher than the commonly quoted dose for cats of 13.2ug/kg/day (Rosychuk, 1982), but appeared to be necessary because a number of cats developed recurrence of alopecia at lower dosages.

In dogs, the half-life of T3 is considerably shorter than T4 (see Section 3.6) and as a result, Nachreiner (1981b), cited by Rosychuk (1982), has recommended administering T3 on a three-times-daily regime. However, Rosychuk (1982) has reported successful maintenance of hypothyroid dogs by twice-daily administration of T3. Reducing the frequency of drug administration is likely to increase owner compliance and in the present series, a twice-daily regime was found to be satisfactory in the majority of cases.

All FEA cases were treated with T3 for 12 weeks before taking further blood samples to monitor progress. This allowed the establishment of a steady state situation for thyroid hormones and a clinical assessment of the response to therapy. No figures are available for cats for the length of time which should elapse before biochemical re-evaluation, following the institution of thyroid hormone therapy, but Rogers, Donovan and Kociba (1975) have suggested a minimum of four to six weeks in dogs. Muller, Kirk and Scott (1983) have recommended that the treatment of canine hypothyroidism should be maintained for a minimum of 12 weeks before making a clinical assessment of its effectiveness.

The serum thyroid hormone concentrations during treatment were assessed from blood samples taken three hours after the administration of half the daily dose of T3. While no figures are available for cats, this regime has been recommended for therapeutic monitoring of hypothyroid dogs treated with T3 (Rosychuk, 1982).

Retrospectively, the FEA cases could be divided according to their response to T3. There was no obvious difference in the age at onset between the groups. However, while equal numbers of neutered females and neutered males were present in the T3 totally responsive

group, neutered females predominated in the other two, comprising 75 per cent and 100 per cent of the T3 partially and non-responsive groups respectively. The significance of these observations is unknown.

In the T3 totally responsive group, the response to treatment was rapid with some hair growth being evident after a mean of four weeks. By comparison, a mean of ten weeks was required for the T3 partially responsive group to show signs of hair regrowth.

A maintenance dose of between 60ug and 100ug T3 was required to prevent thinning of the coat in all but three animals. One of these (108276) was treated when the alopecia recurred (approximately every six months) with 100ug T3 daily until regrowth of hair was complete. Two other cases did not require continuous T3 therapy. One of these (case 130196) had had symptoms for 11 months prior to referral which were unresponsive to dessicated thyroid (dose unspecified). After treatment with 100ug T3 daily for four months, the dose was gradually reduced and subsequently discontinued after a further seven months. In a follow-up period of four years, there was no recurrence of alopecia. In the second case (131365), FEA of five months' duration was treated with T3 for four months. Subsequently, no further treatment was required in a follow-up period of two years. The explanation for these interesting responses is unknown.

Two of the T3 partially responsive cases showed an improvement which was satisfactory to their owners and alternative therapy was not attempted. One other responded totally to depot testosterone. The remaining cat subsequently responded well to oral megestrol acetate therapy.

Two of the T3 non-responsive group were treated with T4 but also failed to respond. Of these, one was subsequently lost to follow-up while one responded completely to depot testosterone. The owner requested euthanasia in the third animal and would not allow a post-mortem examination.

Comparison of physical and laboratory findings in the three groups of feline endocrine alopecia. When the basal physical and laboratory findings of the three groups were compared, the only significant difference noted was between the T4 concentrations. The results suggest that the more T3 responsive an FEA cat is, the lower is its T4 concentration. A similar trend was noted for T3 but this was not statistically significant.

When the laboratory findings 12 weeks after beginning T3 treatment were compared, interesting differences were noted between the T3 totally responsive and the T3 partially and non-responsive groups taken together. The T3 and T4 concentrations were significantly higher and lower respectively during treatment with T3 in the T3 totally responsive group. The low serum T4 concentration would be expected due to the negative feedback of T3 on TSH production. For the T3 partially and non-responsive groups taken together, only the T4 concentration was significantly different before and after treatment. Thus, in this latter group, while the exogenous T3 was at a high enough dose to cause suppression of endogenous T4 production, it was not sufficient to produce a statistical difference between pre and during-treatment T3 concentrations.

Similarly, while the serum cholesterol concentration of the T3 totally responsive group during treatment was significantly lower

compared with the pre-treatment values, such a difference was not noted for the T3 partially responsive and non-responsive groups taken together. Thyroid hormones are known to lower the serum cholesterol concentrations of hypothyroid and euthyroid humans (Anon., 1951; Kritchevsky, 1958) and successive falls in the serum cholesterol concentration of hypothyroid dogs during thyroid hormone therapy is a good prognostic sign (Bush, 1984).

The biochemical differences between the T3 totally responsive group and the T3 partially and non-responsive groups taken together may have a number of explanations. It is possible that T3 absorption was poorer in the latter group and that with a higher T3 dosage a more satisfactory clinical response would have been obtained. The results do not necessarily imply that the condition in either group was the result of hypothyroidism, as Gunaratnam (1986) reported that T4 increases the number of hair follicles entering the anagen phase of the hair cycle in healthy dogs when administered orally or topically.

Alternatively, it is possible that the aetiology of FEA is multifactorial, with the alopecia in the T3 totally responsive group being a manifestation of low thyroid reserve, and in the T3 non-responsive group (and possibly the partially responsive group also), a reflection of extrathyroidal disease. A thyroid biopsy from cat 130985 showed no histological abnormality which suggests that primary hypothyroidism was not the cause of the condition in this animal. Thyroid biopsies were not carried out on any of the animals in the T3 totally responsive group.

Further investigation of the thyroid function of cats with FEA was subsequently carried out by means of the TSH stimulation test (see Chapter 13).

Cases resembling feline endocrine alopecia

The physical signs of FEA are common to a number of diseases. As there is, at present, no definitive diagnostic test for FEA, careful elimination of other diseases must be made prior to beginning hormonal therapy.

Historical and physical parameters. Sixteen per cent of the non-FEA cats seen were entire compared to only 3.7 per cent of the FEA animals. The age at onset was similar in both groups.

No significant variation in the frequency of month of onset of alopecia in the non-FEA group was identified. However, the monthly distribution of onset of the condition in this group was similar to the FEA group and corresponded approximately to the period of minimum hair root activity in cats in the northern hemisphere. The telogen hair root is poorly anchored (Ihrke, 1982). Thus, it might be expected that hair loss would be more severe in any skin condition during this stage of the hair cycle.

Although six of the 25 animals showed lesions of areas other than FEA predilection sites, the lesion distribution in the remainder was very similar to the FEA group. However, 56 per cent of the non-FEA cats showed fractured hairs and 40 per cent accompanying skin changes, whereas these changes were rare in FEA. Pruritus was present at some stage in the disease process in 64 per cent of this group compared with only 3.9 per cent of the FEA cases. However, the degree of pruritus was very variable and frequently the presence of the symptom was only detectable from the history and not on physical examination.

Differential diagnosis of feline endocrine alopecia. In this series, fleas or their faeces were found on nine cats and these

cases responded within six weeks to weekly sprays with a dichlorvos/fenitrothion combination and environmental flea control. Four of these animals had elevated (greater than $1.5 \times 10^9/l$) blood eosinophil counts. In addition, ten cats with no macroscopic evidence of flea infestation responded completely to the same therapy. Of these, five had elevated and three high normal (greater than $1.1 \times 10^9/l$) blood eosinophil counts. As all were negative for ectoparasites on skin scrapings, and for endoparasites on faecal examination, and as neither dichlorvos nor fenitrothion have known stimulatory action on hair growth, it was concluded that these dermatoses were associated with flea infestation. One animal had miliary dermatitis with eosinophilia. This was diagnosed as probable flea infestation but was lost to follow-up.

One cat with flea infestation also showed telogen defluxion which in this and one other case occurred post-partum (Figs. 12.15(a), 12.15(b) and 12.15(c)). Telogen defluxion (also termed telogen effluvium or telogen defluvium) occurs in situations in which the anagen phase of the hair cycle is shortened, resulting in large numbers of hairs entering the resting phase synchronously. The condition may occur at times of physiological stress such as gestation or lactation, in numerous disease states and with certain drug treatments (Thoday, 1986). Affected hairs are easily epilated both by friction and by normal grooming. Specific treatment for the hair loss is not required.

In this series there was a single case each of psychogenic alopecia, pediculosis and mastocytosis.

The case of psychogenic alopecia occurred in a domestic short-haired cat and no initiating cause was identified. Although the

distribution of hair loss was the same as in FEA, remaining hairs in affected areas were fractured and could not be easily epilated. Scott (1980), reviewing the literature on psychogenic alopecia, reported that any breed might be affected but pedigree cats appeared to be predisposed. Stress factors often appeared to precipitate the condition which might have a similar distribution to FEA. However, in psychogenic alopecia, hairs do not epilate easily and appear broken off when examined microscopically (Muller, Kirk and Scott, 1983).

The case of pediculosis (133477) had a similar distribution to FEA (Figs. 12.16(a) and 12.16(b)). Hairs over the trunk were not fractured but there was hyperaemia and self-inflicted injury around the base of the pinnae. Louse infestation in the cat, with lesions compatible with a diagnosis of FEA, does not appear to have been reported previously.

The case of multiple mast cell tumours showed a similar distribution to FEA but the median raphe of the upper lip was also involved. There was diffuse partial alopecia over affected areas but, in addition, there were multiple areas of dry scale, hyperpigmentation and, subsequently, small, variably-sized (2mm to 4mm) papules which distinguished the case from FEA.

Other conditions, not seen in the present study, which may have a similar distribution pattern and lesions to FEA include megestrol acetate-induced diabetes mellitus (Moise and Reimers, 1983), demodicosis (Scott, 1984) and dermatophytosis (Thoday, 1986). Blakemore (1975), cited by Kirk (1980), reported that cats in oestrus may shed their hair easily, particularly in the groin. It is likely that this is merely a manifestation of telogen defluxion.

Comparison of laboratory findings in feline endocrine
alopecia and clinically similar conditions

A number of significant differences in laboratory parameters were identified between the FEA and non-FEA groups. The significance of the lower RBC count and PCV in the non-FEA groups is unknown because this is the converse of what might have been expected if FEA is due to hypothyroidism. A higher serum cholesterol in FEA cats compared to non-FEA animals would be consistent with a tendency towards mild hypothyroidism in the former group, but this is a relatively non-specific feature. In dogs, hypercholesterolaemia may result from feeding high fat diets, hepatopathies, the nephrotic syndrome, acute pancreatitis, diabetes mellitus and hyperadrenocorticalism, in addition to hypothyroidism (Rogers, 1977). Indeed, only four cats in the FEA group were actually hypercholesterolaemic.

The most significant laboratory difference between the two groups was the elevation of the total eosinophil count in the non-FEA group. Eosinophilia may occur in a number of conditions represented in this group. Between 13 and 20 per cent of dogs and cats with flea allergy dermatitis are reported to have blood eosinophilia (Baker, 1974d; Kristensen and Kieffer, 1978; Kieffer and Kristensen, 1979). However, while flea infestation was confirmed or suspected in the present series, affected animals did not exhibit the well-recognised distribution and signs of flea-bite hypersensitivity and the percentage of cases with blood eosinophilia was much higher than that quoted above.

The single case of multiple mast cell tumours had a marked blood eosinophilia. This might have been expected as blood

eosinophilia occurs in a number of diseases involving degranulation of mast cells (Schalm, Jain and Carroll, 1975a).

It follows from the data presented here that, at present, the most useful screening test for the differentiation of FEA and clinically similar conditions is the total blood eosinophil count. Assuming that the prevalence of FEA and non-FEA conditions in this study is the same as in the general population, a raised eosinophil count is highly reliable in excluding FEA. A normal result is of little value in making the diagnosis and is equally probable in FEA and non-FEA conditions.

13. THE THYROID STIMULATING HORMONE STIMULATION TEST IN THE ASSESSMENT OF FELINE THYROID FUNCTION

13.1 GENERAL INTRODUCTION

The TSH stimulation test has been used in the investigation of suspected hypothyroidism in both man and the dog. Various regimes have been reported to give satisfactory responses in healthy cats (see Section 3.9) but there are only a small number of reports of the use of the test in specific diseases in cats (Scott, 1975b, 1980; Peterson, 1982; Peterson et al., 1983b, Arnold et al., 1984; Peterson, 1984).

This chapter details the optimisation of the TSH stimulation test in healthy cats and its subsequent application to the further investigation of FEA, experimental hypothyroidism and naturally-occurring hyperthyroidism.

13.2 REAGENTS

Thyroid stimulating hormone

Bovine TSH was as described in Section 11.4.

13.3 EQUIPMENT

Equipment used in the RIA of T4 and T3 was as described in Section 5.3.

13.4 PRELIMINARY INVESTIGATIONS INTO TSH STIMULATION TESTING IN HEALTHY CATS. (i) DETERMINATION OF THE CHANGE IN SERUM IODOTHYRONINE CONCENTRATIONS WITH RESPECT TO TIME

Methods

Three domestic short-haired cats were used in this experiment. Each was classified as healthy on the basis of a detailed physical examination. Samples were collected from conscious animals by jugular venepuncture at 08.50 hours after starvation from 21.00 hours the previous evening. At 09.00 hours, 5 iu TSH were injected into a cephalic vein. Blood was subsequently collected by jugular venepuncture at one, two, four, six, eight, ten, 12, 16 and 24 hours after TSH administration. After clot retraction, blood was centrifuged and the serum transferred to clean tubes for storage at -40°C. T4 and T3 were assayed on each specimen as described in Chapter 6.

Results

The results of this study are presented in Table 13.1.

Thyroxine concentrations. T4 concentrations showed no significant increases until four hours. In all three cats, the peak T4 concentration was found at eight hours after TSH administration although each had doubled the basal concentration at six hours. At 24 hours, T4 concentrations in cats A and TSH/NC/1/84 approached the basal concentration but remained elevated in cat B.

Triiodothyronine concentrations. As with T4, T3 concentrations showed no appreciable rise until four hours. Peak concentrations were obtained at eight hours after TSH administration. Cat A failed to double its basal T3 concentration during the test.

Discussion

When the present study was carried out, there were no published reports comparing the serum T4 response in cats to varying doses of TSH. However, a number of authors (eg. Scott, 1981) have reported that in healthy dogs, irrespective of weight, 5 iu of TSH administered intravenously causes more than a doubling of basal T4 concentrations four hours after administration. This dose of TSH was therefore selected for initial studies in cats.

As a consequence of this dosage regime, there was considerable variation in the number of units of TSH/kg administered, such that cat A received more than twice the number of units with respect to weight than cat TSH/NC/1/84. However, although both cats' basal T4 concentrations were similar, a much greater increase in serum T4 was seen in cat TSH/NC/1/84, suggesting that the response to TSH is not simply a function of the administered dose.

In contrast to the response in dogs, none of the three cats showed a doubling of their basal T4 concentrations until six hours after TSH administration. The maximal response was seen after eight hours.

Scott (1975b, 1980) also used 5 iu TSH to carry out the TSH stimulation test in healthy cats. Although he administered it subcutaneously and collected his second sample at 12 hours, the degree of response was the same as in the present study. Reimers (1982) and cited by Randolph and Jorgensen (1984) also used 5 iu TSH subcutaneously, but from the data reported, it is difficult to compare his results with the present study.

The changes in serum T3 concentration after TSH stimulation were similar to those described for T4 with maximal concentrations

being seen after eight hours. However, cat TSH/NC/1/84 failed to double its basal T3 concentration. There are no previous reports of the serum T3 response to TSH in cats.

13.5 PRELIMINARY INVESTIGATIONS INTO TSH STIMULATION TESTING IN HEALTHY CATS. (ii) DETERMINATION OF THE OPTIMUM DOSE

Introduction

Dosages of TSH that have been reported to give a satisfactory rise in serum T4 concentrations in healthy cats range from 1.0 iu per cat (Taurog, Porter and Thio, 1964) to 1.0 iu/0.45 kg (Ling, Lowenstine and Kaneko, 1974). However, Morris and Garcia (1983) and Held and Oliver (1984) have demonstrated that 5 iu TSH is sufficient to elicit a satisfactory increase in T4 concentrations in healthy horses, suggesting that much lower doses than have been used previously might be satisfactory in cats. Because TSH is relatively expensive, there has been some reluctance on the part of British veterinary surgeons to use the TSH stimulation test for the diagnosis of canine hypothyroidism. The following study was carried out to determine the optimum dose of TSH for use in the TSH stimulation test in cats.

Methods

The study was carried out as described in Section 13.4 with the exception that the dose of TSH used was 1 iu per cat and that sampling was carried out prior to, and six, eight and ten hours after, TSH administration. To assist in comparing the results, the investigation was carried out on the animals used in the previous study.

Results

The results of this study are presented in Table 13.2 and compared with the previous experiment in Tables 13.3, 13.4 and 13.5.

Thyroxine concentrations. Two of the cats showed peak T4 concentrations at six and one at eight hours after TSH administration. All three animals showed a doubling of basal T4 concentrations at six hours.

Triiodothyronine concentrations. In contrast to the results with T4, one of the cats showed peak T3 concentration at six, and two at eight, hours after TSH administration. Cats A and B showed a doubling of basal T3 concentrations at six hours whereas cat TSH/NC/1/84 just failed to do so. The T3 response to TSH appeared to be more sustained than the corresponding T4 response.

Comparison of the two dosage regimes

Thyroxine concentrations. The T4 responses with respect to time were very similar for the two dosage regimes. However, in all cats, the post-TSH T4 concentration was slightly lower with 1 iu than with 5 iu TSH, but this might have resulted from the lower basal T4 concentrations in two of the cats in the 1 iu study. Using 1 iu TSH, the absolute increase in T4 concentration at six hours was either equal to, or lower than, when using 5 iu. Although samples were only taken up to ten hours after TSH dosing with the 1 iu regime, it appeared that the response with 5 iu was more sustained than with 1 iu.

Triiodothyronine concentrations. The results of the two studies were similar although, surprisingly, there was a tendency for a greater absolute increase in T3 with the lower TSH dosage.

The post-stimulation T4/T3 ratio varied with the TSH dose and the time elapsed after administration.

Discussion

The administration of 1 iu TSH to cats in this study resulted in a dose of between 0.15 iu/kg and 0.34 iu/kg. This produced a satisfactory rise in both serum T4 and T3. Two of the three animals showed peak T4 concentrations at six hours and one at eight hours whereas the reverse was true for T3. Using similar dosage regimes, Hoenig and Ferguson (1983) obtained comparable results for T4, although all six cats they studied with TSH doses of greater than 0.05 iu/kg showed peak concentrations at six hours.

The T3 response of cat A at eight hours was unexpectedly high. When retested, the sample gave the same result. The reasons for this are unclear.

A comparison of the two TSH dosage regimes shows a marginally better T4 response with 5 iu/kg, but the difference is relatively small compared with the five times increase in the amount of TSH used. However, the absolute increase in T3 concentration at six hours with the 1 iu/cat dose was equal to or greater than that seen with 5 iu/cat (equivalent to between 0.78 iu/kg and 1.72 iu/kg). There is no obvious explanation for a poorer response of serum T3 being obtained with a higher dose of TSH. It is probable that a maximal response was obtained with the lower dose and that the difference between the two merely reflects test variables. No previous studies have been published describing the serum T3 response to TSH in healthy cats. However, Faber, Friis, Kirkegaard, Lauridsen, Nerup, Rogowski and Siersbaek-Nielsen (1976) reported that, in man, the maximum response of serum T4 and T3 was

obtained with 0.1 iu/kg TSH, a figure comparable to the 1 iu per cat dose used in the present study.

Analysis of data showed no correlation between the T4/T3 ratio 6 hours after TSH stimulation and the TSH dose, in disagreement with the findings of Faber et al. (1976) in man, who reported that this ratio remained unaltered with increasing doses of TSH.

The data presented here demonstrate that a satisfactory T4 and T3 response can be produced in normal cats at six or eight hours with a dose of 1 iu TSH per cat (equivalent to 0.15 to 0.34 iu/kg). However, as Einhorn and Larsson (1959) commented with respect to man, the results obtained in subjects without thyroid disease should not, without extensive clinical trials, be applied to patients with thyroidal disorders. From the results of the present study, a more satisfactory dose would be 0.35 iu/kg as this produced more than a doubling of the basal T4 concentration in all cats studied. However, this is a difficult figure to compute for injection and a dose of 0.5 iu/kg was selected for further studies. This is half the figure suggested by Hoenig and Ferguson (1983) and is therefore cheaper and consequently more applicable to use in veterinary practice. Using this regime, resampling could be carried out at six or eight hours with satisfactory results. In a clinical situation, sampling at zero and six hours is more convenient and this protocol was used in subsequent investigations.

13.6 THYROID STIMULATING HORMONE TESTING IN HEALTHY CATS - POPULATION STUDIES

Introduction

The pilot studies carried out as described in the previous sections of this chapter suggested a suitable regime for the feline

TSH stimulation test. This study describes the use of the test in healthy cats.

Methods

Sixteen pet cats were used in this study. Prior to selection, each was classified as healthy on the basis of a detailed history and physical examination.

Animals were starved from 21.00 hours the evening before testing. Blood was obtained from conscious animals by venepuncture at 08.50 hours and 0.5 iu/kg TSH solution was injected into a cephalic vein at 09.00 hours. Animals were resampled six hours later by jugular venepuncture. Serum was subsequently collected and stored as described in Section 13.4 and assayed for T4 and T3 as described in Chapter 6.

Results

The results of this study are presented in Table 13.6.

Thyroxine concentrations. The basal T4 concentrations were within the 95 per cent range for healthy cats established previously (see Chapter 7).

All cats showed an obvious T4 response to TSH, with the mean T4 increment being approximately three times the basal concentration. Only one cat (TSH/NC/13/84) failed to double its basal serum T4 concentration six hours after dosing. Linear regression analysis failed to establish a relationship between the increment in serum T4 concentration after TSH administration and the basal T4 concentration ($r = 0.183$, 14 d.f.).

Triiodothyronine concentrations. The basal T3 concentrations were within the 95 per cent range for healthy cats established previously (see Chapter 7).

The T3 response to TSH was much more variable than with T4, ranging from a fall in serum T3 concentration to an almost five-fold increase after six hours. The mean T3 concentration showed an increase of approximately two-fold over basal concentrations. Linear regression analysis failed to establish a relationship between the increment in serum T3 concentration after TSH administration and the basal T3 concentration ($r = -0.035$, 14 d.f.).

Discussion

The development of reliable assay techniques for the determination of thyroid hormone concentrations has simplified the evaluation of thyroid function in veterinary medicine. However, the measurement of basal T4 and T3 concentrations may not always accurately reflect thyroid status in the face of chronic disease, other hormonal abnormalities, drug administration and starvation (reviewed in Section 3.6). The TSH stimulation test has therefore been used in both man and animals to supplement the information available from serum T4 and T3 determinations.

With the regime described here, the minimum total T4 concentration six hours after TSH administration was 56.8 nmol/l with a minimum T4 increment of 21.6 nmol/l. Hoenig and Ferguson (1983) reported that these parameters correlated more closely with the logarithm of the TSH dose than did the ratio of post-TSH serum T4 concentration with baseline T4 concentration. Using the same regime as in the present study but twice the TSH dose, their results (51.5 nmol/l and 32.3 nmol/l respectively) were closely comparable to those found in this study.

The serum total T3 response to TSH was considerably less uniform than the T4 response. One cat showed a marginal fall in

serum T3 concentration after stimulation and three others showed a rise of 0.1 nmol/l or less. This poor response was not related to the basal T3 concentration. There are no previous reports of T3 response to TSH in healthy cats. However, in dogs, T3 responses to TSH are not a reliable index of thyroid function (Nesbitt, 1983), and this is probably because 37 per cent to 60 per cent of T3 is derived from extra-thyroidal mono-de-iodination of T4 (Belshaw et al., 1974). Therefore, the wide variation in T3 response to TSH in this study was not surprising.

The cat showing the lowest T4 response to TSH also showed the poorest T3 response. This animal, a 12-year-old cat, was the oldest in the study. In a follow-up period of 18 months, the animal has remained well and it is interesting to speculate that its poor thyroid reserve was simply associated with ageing.

Of the sixteen animals used in this study, only one showed evidence of toxic reactions to TSH administration. This cat (TSH/NC/1/84) was used in each study. No side-effects were noted during the first two stimulation tests but on the third occasion, the cat became depressed within 30 minutes of TSH injection, and remained so for 48 hours. The animal was anorexic for 24 hours and inappetant for a further 24 hours but returned to normal subsequently. Toxic effects of TSH have not been recorded previously in cats. However, in dogs, repeated intravenous injections of TSH have been reported to produce anaphylaxis (Scott, 1982b) and Muller, Kirk and Scott (1983) advise against the intravenous route if repeated injections have to be given. In the present study, this was not possible and treatment for the reaction in the cat under discussion was not necessary. Taunton,

McDaniel and Pittman (1965) reported the toxic reactions to TSH in 87 human euthyroid patients to be nausea and vomiting (9 patients), tachycardia, nervousness and increased excitability (2), acute pain and tenderness of the neck over the thyroid (3), urticaria (2) and local soreness at the injection site (87). No comparable reactions were seen in the cats under discussion.

Changes in serum thyroid hormone binding proteins can alter baseline and post-TSH T4 concentrations (Hoenig and Ferguson, 1983). Thyroid hormone protein binding studies were not carried out on the sera from animals in this study but all had T4 and T3 concentrations within the respective reference ranges.

The data indicate that, in euthyroid cats, intravenous administration of TSH at a dose of 0.5 iu/kg bodyweight should lead to a T4 concentration of greater than 55 nmol/l at six hours after administration, or to an increment in the serum T4 of greater than 20 nmol/l. The changes in serum T3 concentration in response to TSH are not consistent enough to be used in a clinical situation.

13.7 THE THYROID STIMULATING HORMONE STIMULATION TEST IN FELINE ENDOCRINE ALOPECIA

Introduction

A number of authors have stated that FEA is either a manifestation of hypothyroidism or responds to treatment with thyroid hormones (see Chapter 12). However, Scott (1975b), in the only study of FEA to include TSH stimulation testing, reported that this and other parameters of thyroid function were normal in all animals investigated. In the previous chapter, no definitive laboratory evidence of thyroid abnormalities was found in 26 cases

of FEA investigated in detail. However, 70 per cent of affected animals showed a total response to T3 supplementation.

In order to investigate these apparently contradictory results and reports, the TSH stimulation test was used to assess the thyroid functional reserve in a number of cases of FEA.

Methods

The TSH stimulation test was carried out on six cats which fulfilled the criteria for the diagnosis of FEA as described in Chapter 12. Three cases were totally, and two partially, responsive and one was non-responsive to treatment with T3. Prior to carrying out the tests, in cases receiving thyroid hormone replacement therapy, T3 was discontinued for two months and serum thyroid hormone concentrations were determined to ensure there was no residual therapeutic suppression and elevation of serum T4 and T3 concentrations respectively.

The TSH stimulation test was carried out and the sera harvested and stored as described in Section 13.6. Serum total T4 and T3 concentrations were determined as described in Chapter 6.

Results

The results of this study are presented in Table 13.7.

Thyroxine concentrations. The basal T4 concentrations of the six FEA cats were within the normal range established for healthy cats in Chapter 7 but four were below the range of the 16 healthy cats used in the TSH stimulation tests. The serum T4 concentration and the absolute increase in serum T4 concentration, after TSH administration, were all below the mean values for normal cats (Section 13.6). One cat had a T4 concentration post-TSH marginally below the range found in the normal group. When these parameters

were compared for FEA and healthy animals using the Mann-Whitney U-test, significant differences were identified (Table 13.8). The ratio of post-TSH serum T4 concentration to basal serum T4 concentration was not significantly different between the two groups.

Triiodothyronine concentrations. The basal T3 concentrations of the FEA cats were also within the normal range as established in Chapter 7 but one cat showed a basal T3 lower than the range established for the 16 healthy cats used in the TSH stimulation test.

Using the Mann-Whitney U-test, no significant differences between healthy and FEA cats were identified for basal T3 concentrations, post-TSH T3 concentrations, the absolute increase in serum T3 concentrations after TSH administration and the ratio of post-TSH serum T3 concentrations to basal T3 concentrations (Table 13.8).

Discussion

The basal T4 concentrations of all six FEA cats used in this study fell into the reference range derived from observations in 318 animals (see Chapter 7). However, when compared to the basal T4 concentrations of the 16 animals used for the optimisation of the TSH test in healthy cats (Section 13.6), four FEA cats had subnormal basal T4 concentrations. It is possible that this resulted from the smaller sample size of healthy animals used for the TSH study. Between-assay variation in the T4 and T3 assays may also have contributed to the observed pattern of results.

Scott (1975b) is the only person to have reported the results of TSH testing in FEA. Five affected cats showed a normal response to

TSH, (defined as a doubling or tripling of the basal T₄ concentration), 12 hours after the subcutaneous administration of 5 iu TSH. Detailed statistical analysis of the results was not reported.

The serum T₃ response to TSH in cats has not been reported previously. In this study, there were no significant differences between the FEA and the healthy cats in any of the TSH stimulation test parameters for T₃ measured. As was discussed in Section 13.6, the serum T₃ response to TSH is not a reliable indicator of thyroid function and the present findings were anticipated.

Although a more extensive study is required before any definite conclusions can be drawn, the present data suggest the possibility that FEA in some cats may be a manifestation of low thyroid reserve. This term was originally used by Jefferies et al. (1953) to describe a condition of man characterised by a normal three-hour thyroidal uptake of ¹³¹I and serum PBI concentration but a failure of either to respond to stimulation with TSH. They attributed the condition to the presence of a remnant of thyroid tissue which was being stimulated maximally by endogenous TSH and hence could not respond further to the exogenous hormone administered in the test. Subsequently, Levy, Kelly and Jefferies (1954) observed that in some circumstances ¹³¹I uptake would rise following administration of TSH but the serum PBI concentration would not, whereas in others the reverse occurred. Low thyroid reserve in humans may be a transient phenomenon or may persist indefinitely, as may FEA.

Low thyroid reserve was observed more frequently in patients who had had ¹³¹I therapy or subtotal thyroidectomy, but was also

seen following thyroiditis and within three to eight weeks of cessation of prolonged thyroid hormone medication. In the present study, T3 therapy was discontinued for a minimum of eight weeks prior to TSH testing in all cats. Four had had no other drug treatments in that time. One had received diazepam from the referring veterinary surgeon for its FEA. There appear to have been no reports of diazepam causing decreases in serum thyroid hormone concentrations or response to TSH. One animal had received a depot injectable preparation of testosterone esters, (Durateston, Intervet Laboratories, duration of action of one month), two months before testing, as it was non-responsive to T3. Androgens have been reported to lower T4 and T3 concentrations in dogs but in such cases, the T4 response to TSH parallels that seen in untreated, healthy individuals (Ferguson, 1984). However, this is unlikely to have influenced the test results in the case in question as therapy was effectively discontinued one month before testing.

Because ^{131}I uptake studies were not carried out, it is not possible to suggest whether the cats in the present study might be classified into similar sub-groupings of low thyroid reserve as described in man. Indeed, it is interesting to speculate that, because cases of FEA may be subdivided retrospectively according to response to T3, the syndrome may represent a number of thyroidal and non-thyroidal states. Further studies using a combination of ^{131}I uptake and TSH stimulation are warranted in FEA to determine more clearly whether the condition in some animals may be a manifestation of low thyroid reserve.

13.8 THE THYROID STIMULATING HORMONE STIMULATION TEST IN

EXPERIMENTAL FELINE HYPOTHYROIDISM

Introduction

Although attempts have been made to induce hypothyroidism experimentally in cats on a number of occasions (see Chapter 11), TSH stimulation testing has never been employed to confirm the hypothyroid state. This section describes the use of the TSH stimulation test in two cats in which the thyroid glands had previously been ablated with radioiodine.

Methods

The two cats used were those described in detail in Chapter 11. One hundred and ten weeks after the administration of radioiodine, the TSH stimulation test was carried out as described in Section 13.6. Serum was subsequently collected and stored as described in Section 13.4 and assayed for T4 and T3 as detailed in Chapter 6.

Results

The results of this study are presented in Table 13.9.

Serum T4 and T3 were undetectable (<1.3 nmol/l and <0.10 nmol/l respectively) both before and after administration of TSH. In addition, in all test samples, the per cent radioisotope bound was greater than that in the corresponding zero standard for that assay.

Discussion

These experimental cats had shown serum T4 and T3 concentrations consistently below the detection limits of the respective assays for 107 weeks prior to TSH stimulation testing. The lack of any obvious T4 or T3 response to TSH provides clear confirmation that thyroid ablation in these animals had been

successful. Repeated TSH administration is not required for the demonstration of primary hypothyroidism by thyroid hormone assay.

There are no previous reports of the confirmation of experimental feline hypothyroidism by TSH testing. Arnold et al. (1984) are the only workers to have substantiated a diagnosis of naturally-occurring feline hypothyroidism by TSH stimulation. They measured serum T4 concentrations before and ten hours after the last of three, consecutive, daily, intramuscular injections of 5 iu bovine TSH to a 14-week-old kitten with suspected goitrous hypothyroidism and dwarfism. The T4 concentration was below the detection limit of the assay (0.4 nmol/l) on both occasions.

A case of naturally-occurring, acquired, feline hypothyroidism has yet to be definitively diagnosed. The present study illustrates the potential value of the TSH stimulation test in the investigation of possible cases.

13.9 THE THYROID STIMULATING HORMONE STIMULATION TEST IN FELINE HYPERTHYROIDISM

Introduction

Hyperthyroidism is a multisystemic disorder, resulting from excessive circulating concentrations of T4 and T3. Although a TSH stimulation test is not required for the diagnosis of hyperthyroidism in most affected cats, it has been suggested that it might be of value when resting thyroid hormone concentrations are borderline or only slightly increased (Peterson, 1982; Peterson et al. 1983b; Peterson, 1984). This section reports on the use of the TSH stimulation test in a single case of feline hyperthyroidism.

Methods

Case details of the patient are presented in Table 13.10.

When 12 years old, the cat was presented with a history and clinical signs suggestive of hyperthyroidism and the diagnosis was confirmed by the demonstration of elevated serum total T4 and T3 concentrations. Bilateral thyroidectomy resulted in total remission of clinical signs and laboratory tests returned to normal. Histopathological examination of the thyroid revealed bilateral adenomatous hyperplasia. Both glands were infiltrated with lymphocytes. A serum sample taken on initial presentation was scored as ++ for thyroid microsomal antibodies (see Chapter 14). Ninety-two weeks after surgery, the cat was represented with a recurrence of clinical signs and hyperthyroidism was confirmed by the demonstration of elevated serum total T4 and T3 concentrations. TSH stimulation testing was carried out at this stage, as described in Section 13.6. Serum was subsequently collected and stored as described in Section 13.4 and assayed for T4 and T3 as detailed in Chapter 6.

Results

The results of the TSH stimulation test are presented in Table 13.10.

The absolute increase in serum T4 concentration after TSH administration and the ratio of post-TSH serum T4 concentration to basal serum T4 concentration were within the respective ranges for normal cats. The absolute increase in serum T3 concentration after TSH administration was above the normal range, but the ratio of the post-stimulation to basal concentrations was normal.

Discussion

The normal responses to TSH stimulation found here in a single animal do not support the conclusions of Peterson (1982), Peterson et al. (1983b) and Peterson (1984) who suggested that the lack of serum T4 response to TSH might be helpful where a diagnosis of feline hyperthyroidism is equivocal. They showed that four hours after the intravenous administration of 1 iu/kg TSH, the mean serum T4 concentration of 11 hyperthyroid cats (144.1 nmol/l) was not significantly higher than the mean basal T4 concentration (127.1 nmol/l). T4 concentrations increased by more than 19.3 nmol/l above baseline concentrations in only three cats, with the highest increase being 87.5 nmol/l (Peterson et al. 1983b). The authors suggested that either the thyroid glands of thyrotoxic cats secreted thyroid hormones independently of TSH control, or that T4 and T3 were already being produced at a maximal rate. These alterations would correspond to human toxic multinodular or nodular goitre and Graves' disease.

Although only one animal was studied in the present report, it is clear that the lack of serum T4 response to TSH has limitations for the diagnosis of feline hyperthyroidism. A sub-normal T4 response may indicate thyrotoxicosis but a normal increase in T4 concentration does not exclude the diagnosis.

There are no previous reports of changes in serum T3 concentrations in response to TSH in feline hyperthyroidism. In the present study, the absolute increase in serum T3 concentration after TSH administration was greater than that seen in any healthy animal studied. As discussed for healthy animals in Section 13.6, the response of serum T3 to TSH is unlikely to be a reliable test of feline thyroid function.

14. AUTOANTIBODY STUDIES IN FELINE NATURALLY-OCCURRING HYPERTHYROIDISM AND RADIOIODINE-INDUCED HYPOTHYROIDISM

14.1 INTRODUCTION

Circulating autoantibodies to various thyroid components have been identified in human hyperthyroidism, both prior to treatment and after thyroid ablation using ^{131}I . Thyroid autoantibodies have also been induced in euthyroid humans by radioiodine therapy (reviewed in Section 3.14). Similar immunological investigations have not been carried out in cats.

This section describes autoantibody studies in cases of naturally-occurring feline hyperthyroidism, and in two cats in which hypothyroidism was induced experimentally using radioiodine.

14.2 REAGENTS

Anti-cat immunoglobulin

Goat anti-cat IgG conjugated with fluorescein isothiocyanate (Miles-Yeda) was obtained from Miles Scientific, Stoke Court, Stoke Poges, England.

Thyroid stimulating hormone receptor assay

Measurement of TSH receptor antibodies was attempted using a kit technique (TSH Receptor Antibody Assay Kit, R.S.R. Ltd., St. Nicholas, Cardiff, South Wales).

14.3 EQUIPMENT

The ultra-violet microscope was a Leitz Ortholux II, obtainable

from E. Leitz (Instruments), Luton, England, and fitted with a Wild MPS 51 camera attachment.

Other equipment was as described in previous chapters.

14.4 METHODS

Examinations for thyroid microsomal, antinuclear and TSH receptor antibodies were carried out in collaboration with Dr. R.L. Kennedy.

Thyroid microsomal and antinuclear antibodies

Thyroid microsomal antibodies and ANA were measured by indirect immunofluorescence on normal cat thyroid.

Thyroid tissue was removed from a healthy cat immediately after euthanasia by intravenous injection of 20 per cent w/v pentobarbitone sodium solution (Euthatal, May and Baker), and frozen in liquid nitrogen.

Sera were diluted 1:4 with phosphate buffered saline (PBS), pH 7.2, and applied to 5µ sections of healthy feline thyroid for 30 minutes at room temperature. The sections were then washed in PBS for 30 minutes with two changes of buffer. Goat anti-cat IgG conjugated with fluorescein isothiocyanate was then applied at a dilution of 1:8 for 30 minutes and the slides were subsequently washed for two hours with several changes of buffer.

The sections were coded and read blindly under ultraviolet light and were scored as follows:

- 0 - No fluorescence
- + - Borderline fluorescence (not counted as positive)
- +
- ++ - Strong positive

The non-organ specific nature of the ANA detected in some cats was confirmed by repeating the experiment using rat kidney as substrate.

Thyroid stimulating hormone receptor antibodies

TSH receptor antibodies were sought in undiluted serum using a kit radioreceptor assay based on the method of Southgate, Creagh, Teece, Kingswood and Rees Smith (1984).

The technique depends on the serum antibody's ability to inhibit ^{125}I -labelled TSH binding to a detergent solubilised preparation of thyroid TSH receptors. Between-assay precision was approximately 12 per cent, two per cent and 2.5 per cent cv at mean inhibition of TSH binding values of 11, 53 and 79 respectively.

Thyroxine and triiodothyronine determinations

Serum total T4 and T3 concentrations were determined as described in Chapter 6.

Histological examinations

Thyroid lobes excised from cases of feline hyperthyroidism were preserved in ten per cent formal saline and processed and examined as described in Chapter 10.

14.5 NATURALLY-OCCURRING FELINE HYPERTHYROIDISM

Clinical material

This study comprised 29 consecutive cases of feline hyperthyroidism. The tentative diagnosis was made on the basis of history and clinical findings and confirmed by the presence of elevated total serum thyroid hormone concentrations.

Serum samples were collected by jugular venepuncture, stored at -40°C and tested in batch mode. Indirect immunofluorescence

testing was carried out on sera from all 29 cats and from 15 healthy cats of a wide age range to act as controls. TSH receptor antibody assay was carried out on sera from 12 of the thyrotoxic cats and from 12 healthy controls.

Two affected cats died soon after the diagnosis was made. Euthanasia was requested by the owners in two further cases. One cat was treated medically with PTU.

The remaining 24 cases were treated surgically by unilateral or bilateral thyroidectomy as described in Chapter 10. Thyroid tissue was preserved for immunological studies by snap-freezing in liquid nitrogen and was subsequently stored at -40°C .

Two cats died within two days of surgery. One case was lost to follow-up. Twenty-one cases were followed up intermittently after surgery.

Results

There were 28 domestic short-haired cats and one domestic long-haired cat in the study. Thirteen (45 per cent) were male (one entire and 12 castrated) and 16 (55 per cent) were female (three entire and 13 ovariectomised). The mean age was 12.8 years with a range of six to 17 years. The historical and clinical features were largely in agreement with those of the more extensive series described in Chapter 10 and those published by other workers and are summarised in Table 14.1. Twenty-eight cats had a palpable goitre, with 16 being unilateral and 12 bilateral.

The serum T4 concentration was elevated in all cats but the serum T3 concentration was within the normal range in one animal. In two cats, the serum T4 concentration was not precisely quantified and was reported as greater than 200 nmol/l. In the remainder, the

range for serum T4 concentration was 65.1 nmol/l to 654.0 nmol/l (mean 197.9 nmol/l) and for T3 was 1.06 nmol/l to 10.3 nmol/l (mean 3.87 nmol/l).

Thyroid microsomal and antinuclear antibodies. Thyroid autoantibodies were detected in ten cases, five of which were strongly positive. ANA was identified in another four cats. Serum from all 15 control cats was negative for thyroid autoantibodies and ANA.

Clinical and laboratory data from the 15 hyperthyroid antibody negative cats are shown in Table 14.2, from the ten thyroid antibody positive cats in Table 14.3 and from the four ANA positive cats in Table 14.4.

Thyroid antibodies. Cat thyroid incubated with serum from a healthy cat and serum strongly positive for thyroid autoantibodies is shown in Figs. 14.1 and 14.2 respectively. Thyroid tissue from the thyrotoxic cats was also satisfactory for detection of thyroid antibodies.

The ten thyroid antibody positive cats consisted of six neutered males and four neutered females. There was no significant difference between the ages at diagnosis between the thyroid antibody positive cats (mean of 13.3 years) and the 17 antibody negative cats (mean of 12.5 years) as analysed by Student's unpaired t test.

The mean serum total T4 and T3 concentrations in the thyroid antibody positive cats was 166.0 nmol/l and 3.93 nmol/l respectively compared with 213.8 nmol/l and 3.84 nmol/l in the thyroid antibody negative cats. Because two of the high T4 concentrations were not precisely quantified, the Wilcoxon Rank Sum test was used to

compare these data whereas Student's unpaired t test was used for the T3 concentrations. None of the differences was significant.

Of the five cats scored ++ for thyroid antibodies, four had bilateral goitres compared with only five of the other 22 cats. Lymphocytic infiltrates were found in four of the five thyroids but only in five of the 22 thyroids examined from the rest of the cats. Using Fisher's exact test, these variables were found to be significantly different ($P < 0.05$ in both cases).

Antinuclear antibodies. Cat thyroid incubated with serum positive for ANA is shown in Fig. 14.3.

All four cats positive for ANA had unilateral goitres and two affected thyroid glands contained lymphocytic infiltrates. The two youngest cats in the series, aged six and 7.5 years, were in this group.

The serum total T4 (mean 132.3 nmol/l) and T3 (mean 3.24 nmol/l) concentrations of the ANA positive cats did not differ from those of the other hyperthyroid cats as analysed by the Wilcoxon Rank Sum test and Student's unpaired t test respectively.

Thyroid stimulating hormone receptor antibodies. TSH receptor antibodies were not detected in sera from any of the 12 hyperthyroid or the 12 healthy cats.

Follow-up of cats treated surgically. It was possible to follow up 21 cats after surgery, over a range of three to 40 months, with a mean of 11.1 months. During this time, recurrence of hyperthyroidism was confirmed in three cats (113897, FT/HE/17/83 and FT/HE/15/82) after twenty-one months, seven months and an unspecified period respectively.

Two of these cats (113897 and FT/HE/17/83) had shown strongly positive reactions for serum thyroid antibodies and bilateral goitre at initial diagnosis. After bilateral extracapsular thyroidectomy, lymphocytic infiltrates were found in excised thyroid glands. The third cat (FT/HE/15/82) had shown a strongly positive reaction for serum ANA and unilateral goitre. This cat had been treated by unilateral extracapsular thyroidectomy and lymphocytic infiltrates were found in excised tissue. Recurrence in this case had involved the side on which surgery had already been performed.

FT/HE/17/83 and FT/HE/15/82 responded totally to further surgery and there was no recurrence of the condition. With 113897, a second bilateral thyroidectomy produced remission of clinical signs and lymphocytic infiltrates were again found in the excised thyroid tissue. After a further five months, the cat became thyrotoxic once more. Treatment with PTU controlled the symptoms and signs of the condition but had to be withdrawn after six months because of severe although asymptomatic thrombocytopenia. Further surgery recovered cystic thyroid tissue but clinical and biochemical hyperthyroidism persisted and no further treatment was given.

Discussion

The 29 hyperthyroid cats in this report presented consecutively but were not selected for autoantibody studies by any other criteria. Using normal cat thyroid as tissue substrate, circulating autoantibodies were detected by indirect immunofluorescence in 14 of these cats (48 per cent). Ten showed thyroid antibodies with a staining pattern similar to that of the microsomal antibody found in human thyroid diseases and four had

ANA. The non-organ specific nature of the ANA was demonstrated by incubating the positive sera with rat kidney.

The five sera with strong reactions for microsomal antibody included four cats with bilateral goitre, and four of the five thyroids removed contained lymphocytic infiltrates. Such infiltrates were found in only five of the other 22 thyroids examined, and sera from two of these five affected cats were positive for ANA. The correlation between lymphocytic infiltration of the thyroid gland and circulating antibodies to the thyroid is well-recognised in human thyroid disorders (Schade et al., 1960).

Despite surgical therapy using the technique of extracapsular thyroidectomy, thyrotoxicosis recurred in three cats. Two of these had bilateral goitres with lymphocytic infiltrates at initial presentation and their serum was strongly positive for thyroid antibodies. The third cat had a unilateral goitre but there was lymphocytic infiltration and its serum was positive for ANA. Autoantibodies may therefore have prognostic significance in feline hyperthyroidism as they do in human Graves' disease (Irvine, Gray, Morris and Ting, 1977; McGregor, Rees Smith, Hall, Petersen, Miller and Dewar, 1980).

Recurrence of feline thyrotoxicosis after bilateral thyroidectomy has been recorded previously (Peterson, Birchard and Mehlhaff, 1984). Proposed or proven causes include continued hyperplasia of remnants of thyroid tissue left with the thyroid capsule after intracapsular thyroidectomy (Birchard, Peterson, and Jacobson, 1984; Peterson, 1984), local regrowth of, or distant metastasis from, a thyroid adenocarcinoma (Cotter, 1979; Hoenig et al., 1982; Olsen, 1982, 1983) and involvement of accessory or

ectopic thyroid tissue in the disease process (Holzworth et al., 1980; Olsen, 1982, 1983).

In the three cases under discussion, the thyroid was excised within its capsule. In the cases of bilateral thyroidectomy, it is possible that remnants of thyroid tissue may have remained with the cranial parathyroid glands which were carefully dissected free of the cranial thyroid capsule and preserved. However, the cranial parathyroid gland is situated in an extracapsular position (Section 3.3) and this is therefore unlikely. This cannot be the explanation for the recurrence on the same side of the neck in the unilateral case, as here a unilateral thyroparathyroidectomy was carried out. Neither can recurrence in any of the cases be due to malignancy as this was excluded on histological examination of excised tissue in each.

It is tempting to speculate that recurrence of thyrotoxicosis in these cases was due to involvement of accessory or ectopic thyroid tissue in the disease processes. Alternatively, because hyperfunctional thyroid tissue frequently contains numbers of cysts of varying size, it is possible that spillage of cystic fluid together with viable thyroid cells, resulted in eventual recurrence of hyperthyroidism in these three cats.

In healthy cats, serum ANA has been variously reported as rare (Scott, Haupt, Knowlton and Lewis, 1979) and occurring in low titres (between 1:2 and 1:10) in ten per cent of normal cats (Pedersen, 1982). In a series of 100 healthy cats, ANA was identified in only two, at a titre of 1:5 (D.W. Scott, personal communication). In the present study, sera from all 15 healthy controls were negative for ANA.

Positive serum ANA may occur in cats with systemic lupus erythematosus (Scott et al., 1979; Pedersen, 1982; D.W. Scott, 1984), pemphigus erythematosus (Scott, Miller, Lewis, Manning and Smith, 1980); cholangiohepatitis, leukaemia virus infection and infectious peritonitis (Pedersen, 1982) and fibrosarcoma, (D.W. Scott, personal communication). In addition, as discussed previously, Peterson et al. (1984) identified serum ANA as a consequence of PTU therapy in five hyperthyroid cats. The four cats which were positive for ANA in the present series did not show signs of other diseases and had not been treated with PTU and it is likely that their ANA titres were related to their thyrotoxicosis rather than to other disease states.

In this series, two of the ANA positive cats (aged six and 7.5 years) are among the youngest cases of feline hyperthyroidism yet reported (see Section 3.11). If thyroid autoantibody production is secondary to thyroid damage rather than part of a primary immunological disturbance, the formation of non-organ specific autoantibodies would not be surprising although ANA may also be found in human autoimmune thyroid disease (Hijmans, Doniach, Roitt and Holborow, 1961).

Circulating TSH receptor antibodies were not identified in any animal in this series. This is not surprising as the assay used in this study has been developed for use with human serum.

Histological examination of excised thyroid glands from these cats, as in the larger series reported in Chapter 10, revealed adenomatous hyperplasia in all cases. Feline hyperthyroidism has an equal sex incidence, unlike the female predominance in Graves' disease, and is a disease of older cats (see Chapter 10). The

condition, therefore, has many similarities to human toxic nodular goitre.

Thyroid antibodies are frequently found in cases of human toxic nodular goitre (Codaccioni et al., 1971) and, although it is possible that they may simply reflect tissue damage, a variety of other immunological disturbances have been described (Kiy et al., 1982; Smyth, Neylan and O'Donovan, 1983). An autoimmune aetiology is not, therefore, excluded by the finding of toxic nodular goitre.

The immunological aspects of feline hyperthyroidism have not been studied previously. Many cases have serum autoantibodies although their pathogenic role, if any, remains to be clarified. The aetiology of the condition requires further investigation. Such work may be of relevance, not only to veterinary, but also to medical practice, in an area in which animal models are sadly lacking.

14.6 RADIOIODINE-INDUCED FELINE HYPOTHYROIDISM

Clinical material

Serum samples from two experimental cats (see Chapter 11) before (week 1) and on two occasions after (weeks 18 and 67) thyroid ablation with radioiodine were studied. Samples were collected and stored as described in Chapter 11. Sera were examined by indirect immunofluorescence testing as described in Section 14.4 but TSH receptor antibodies were not assayed.

Results

Clinical and laboratory details of the two cats at the time of sampling, together with the results of direct immunofluorescence testing are given in Table 14.5.

Thyroid antibodies and ANA were not identified in any of the sera tested.

Discussion

Neither thyroid microsomal antibody nor ANA were induced in either cat as a result of thyroid ablation with radioiodine. These results are not surprising because, although microsomal and thyroglobulin antibodies have been induced in low titres in euthyroid humans as a result of ^{131}I therapy, this occurred in only ten and eight patients respectively of 28 studied (Einhorn, Fagraeus and Jonsson, 1966).

Thus, microsomal autoantibody or ANA formation was not contributory to the progressive elevations of gamma-globulin concentrations in the cats in the present study (see Chapter 11). Other thyroid antibodies that have been demonstrated in human thyroid diseases include antithyroglobulin antibody and a second antibody against a colloid antigen distinct from thyroglobulin (Ingbar, 1985). Detection of these antibodies requires techniques which were not carried out in this study. It is unlikely that the degree of increase in gamma-globulin concentrations observed in these cats (approximately 2.5 times the basal concentrations) could be due merely to thyroid autoantibody formation. Similar increases in serum gamma-globulin concentrations occur in human Hashimoto's disease, but these are not due simply to thyroid antibody formation and the exact mechanism is unknown (A.D. Toft, personal communication). Human B cells may be activated by a large number of triggering signals to produce a polyclonal response resulting in elevated gamma-globulin concentrations (Fauci, 1980, 1983). It is interesting to speculate that such B cell stimulation may be the end result of thyroid ablation with radioiodine in cats.

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A P P E N D I X

PUBLICATIONS FROM STUDIES
DESCRIBED IN THIS THESIS

Radioimmunoassay of serum total thyroxine and triiodothyronine in healthy cats: assay methodology and effects of age, sex, breed, heredity and environment.

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ABSTRACT

Double antibody radioimmunoassays for thyroxine (T4) and triiodothyronine (T3) were established for use in cats, using standards in T4- and T3-free, normal cat serum. The assay working ranges were 5–200 nmol/l for T4 and 0.125–10 nmol/l for T3. Recovery of added hormone was 106 per cent \pm 5.9 for T4 and 104 per cent \pm 8.5 for T3 (mean \pm 1 s.d.). Mean between-assay coefficients of variation (c.v.) were 6.6 per cent for T4 and 12.1 per cent for T3.

Serum total T4 levels of 318 and total T3 levels of 299 healthy cats aged 4 months to 13 years were measured. Mean \pm 1 s.d. values were 26.1 \pm 10.1 nmol/l for T4 and 0.69 \pm 0.29 nmol/l for T3. T4 and T3 concentrations within individual animals were highly correlated. T4 levels in both sexes tended to decrease until approximately 5 years of age and then rise again. A similar, though less pronounced effect, was found for T3, concentrations for older cats levelling out rather than rising.

For any given age, females and neutered females tended to have significantly higher T4 values than males and neutered males, but such effects were not significant for T3. When other effects were accounted for, the effects of neutering were not significant for levels of either hormone. Pedigree animals tended to have higher levels of T3 at any given age than domestic short- and long-haired cats taken as one group. Animals living in the same environment had significantly similar T4 levels and T3 levels. For

T3, this appeared to be due to a definite genetic component, but it was not possible to differentiate between environmental and genetic effects for T4.

INTRODUCTION

Thyroid function of cats has been little studied in comparison with that of the dog. This may have resulted, in part, from an apparent lack of thyroid-related disease states in the former. However, with the recent recognition of hyperthyroidism as an important condition of older cats (Peterson, Johnson & Andrews, 1979; Holzworth *et al.*, 1980) and further definition of feline endocrine alopecia as a thyroid hormone-responsive dermatosis (Thoday, 1983), a more detailed understanding of the cat's thyroid physiology is urgently required.

Many of the reported studies of feline thyroid function have used methods which have been superseded in studies on humans because of poor specificity, sensitivity or both. Thus, early studies using serum protein-bound iodine (e.g. Bustad & Fuller, 1970; Kaneko, 1970), triiodothyronine (T3) uptake (e.g. Kallfelz & Erali, 1973; Ling, Lowenstine & Kaneko, 1974), competitive protein binding (CPB) (e.g. Scherzinger & Grosser, 1972; Scherzinger, Guzy & Lorcher, 1972; Kallfelz & Erali, 1973; Ling, Lowenstine & Kaneko, 1974; Bigler, 1976a) and free thyroxine (T4) index (Kallfelz & Erali, 1973) were valuable but failed to provide the accurate information on circulating T4 and T3 levels which may be obtained using radioimmunoassay (RIA) methods now available.

Two studies have reported details of total thyroid hormone levels in normal cats measured by RIA. Reap, Cass & Hightower (1978) measured total T4 and T3 and Anderson & Brown (1979) total T4 and T3 uptake values. The only other reports using RIA have referred to normal ranges without giving information about the numbers and types of animals studied, in clinical papers on feline hyperthyroidism or in review articles. (Peterson, Johnson & Andrews, 1979; Holzworth *et al.*, 1980; Peterson, Becker & Hurley, 1980; Theran & Holzworth, 1980; Jones & Johnstone, 1981; McMillan & Scherding, 1981; Nesbitt, 1981 (quoted in Rosychuk, 1982); Peterson, 1981; Peterson, 1981 (quoted in Rosychuk, 1982); Peterson *et al.*, 1981; Theran, 1981; Watson *et al.*, 1981; Hoenig *et al.*, 1982; Peterson *et al.*, 1982; Reimers, 1982; Belshaw, 1983; Kirk, 1983; Noxon *et al.*, 1983; Peterson *et al.*, 1983; Peterson and Yoshioka, 1983; Peterson & Becker, 1984.)

There is, therefore, a need for a more detailed study of serum thyroid hormone levels in normal cats using specific and accurate techniques and examining possible effects of age, sex, breed, genetic relationships and environment. Such a study is described here.

MATERIALS AND METHODS

Reagents

Sheep anti-T4 and sheep anti-T3 sera were obtained from the Scottish Antibody Production Unit (SAPU), Carlisle, Lanarkshire, Scotland. Donkey anti-sheep

serum (DAS) for use as a precipitating antiserum and normal sheep serum (NSS) were obtained from the same source. T4 sodium salt and T3 free acid for use as standards, and 8-anilino-naphthalene-1-sulphonic acid (ANS) were obtained from the Sigma Chemical Company. ^{125}I -labelled T4, of specific activity > 34.5 MBq/nmol, and ^{125}I -labelled T3, of specific activity > 28.9 MBq/nmol were obtained from the Radiochemical Centre, Amersham, Bucks., England.

T4 and T3 standards

Standards were prepared in hormone-free cat serum to minimize the errors that arise if protein concentrations in standard and sample incubates are unequal. Pooled serum from normal cats was stripped of endogenous hormones by stirring with Dowex 2 - X⁸ (Cl⁻) anion exchange resin (Salter, 1979). The resin-treated serum was separated by filtration rather than decanting to increase the yield. This procedure removed more than 99 per cent of T4 and T3, as measured from the recovery of ^{125}I -labelled hormones. Sodium azide (0.01 per cent) was added to the stripped serum as a bacteriostat.

Stock solutions of T4 and T3 were prepared in alkaline propylene glycol and concentrations were confirmed spectrophotometrically. Appropriate dilutions of the stock solutions in assay diluent were added to hormone-free serum to give standards of 0, 5, 10, 20, 40, 60, 80, 120, 160 and 200 nmol/l for T4 and 0, 0.125, 0.25, 0.5, 1, 1.5, 2, 3, 4, 5 and 10 nmol/l for T3. The standards were stored in aliquots at -40°C .

Radioimmunoassay methods

Serum T4 and T3 were measured by radioimmunoassays based on the double antibody methods described by Ratcliffe, Challand & Ratcliffe (1974) and modified for use with feline serum. The dilutions of anti-T4 and anti-T3 sera were adjusted to give the maximum response over the concentrations anticipated in feline serum. Concentrations of NSS and DAS were optimized by the procedures of Hunter (1978). All determinations were performed in duplicate in polystyrene tubes (12×75 mm). The assay diluent was 0.05 mol/l sodium barbiturate buffer, pH 8.6, containing 0.1 per cent bovine serum albumin and 0.01 per cent sodium azide. The reagents and procedure are summarized in Table 1.

Clinical Material

Blood was obtained from 319 cats (122 males, 141 females, 35 neutered males and 21 neutered females) aged between 4 months and 13 years. All were classified as healthy on the basis of detailed history and physical examination. Pregnancy checks were carried out on all entire females by ballottement and those considered to be positive were excluded from the study. The majority of the animals were either domestic short- or long-haired, but 23 pedigree animals of several breeds were also represented. Samples were collected from conscious animals by venepuncture, usually from the jugular vein but also from the cephalic vein. After

TABLE 1. Constituents of assays and procedures

T4 radioimmunoassay		T3 radioimmunoassay	
Serum (standard or sample)	25 μ l	Serum (standard or sample)	50 μ l
Reagent 1 125 I-T4		Reagent 1 125 I-T3	
approx. 35,000 c.p.m./tube	600 μ l	approx. 30,000 c.p.m./tube	400 μ l
DAS (1:73)		DAS (1:29)	
Reagent 2		Reagent 2	
ANS (290 μ g/tube)	200 μ l	ANS (90 μ g/tube)	560 μ l
Sheep anti-T4 (1:4,850)		Sheep anti-T3 (1:8,400)	
NSS (1:1,360)		NSS (1:680)	
Total volume	825 μ l	Total volume	1,050 μ l

Procedure

1. Using an automatic diluter, pick up standard or sample and dispense together with Reagent 1. Dispense reagent 2. Vortex mix.
2. Incubate for 36 hours at 4°C.
3. Centrifuge at 3,000 r.p.m. for 30 minutes at 4°C.
4. Decant supernatant to waste. Blot tube tops dry on absorbent paper.
5. Determine antibody-bound 125 I in tubes by counting in a gamma counter.
6. Enter count results on paper tape via Hewlett Packard tape reader (Model 9883A) and calculator (Model 9821A) into computer (Hewlett Packard 9871A) for calculation of results using 4 parameter logit-log model for the standard curve.

clot retraction, blood was centrifuged and the serum transferred to a clean tube for storage at -40°C. Where the volume of sample allowed, T4 and T3 were assayed on each specimen giving 298 paired observations. Twenty samples were assayed for T4 alone and one for T3 alone.

RESULTS

Validation of assay procedures

(a) *Elimination of serum interference.* ANS was included in the RIA incubate to block binding of thyroid hormones by serum proteins which would otherwise interfere in the assay. The optimum concentrations were determined by varying the amount of ANS per tube between 0 and 1,500 μ g and selecting the concentration which yielded the minimum difference in binding between incubates containing diluent only and diluent plus hormone-free serum. For T4, the ANS concentration required was 290 μ g/tube. For T3, a lower concentration of ANS was required, possibly reflecting the lower affinity of T3 for serum proteins, 90 μ g/tube being effective.

(b) *Accuracy.* Accuracy was assessed by determining the recovery of known amounts of T4 or T3 added to normal feline sera to give concentrations in the

TABLE 2. Between assay precision for T4 and T3 radioimmunoassays

T4 radioimmunoassay				T3 radioimmunoassay			
Pool	Mean T4 concentration (nmol/l)	sd	c.v. (%)	Pool	Mean T3 concentration (nmol/l)	sd	c.v. (%)
Low	18.08	1.31	7.2	Low	0.29	0.05	17.2
Medium	37.10	2.86	7.7	Medium	0.47	0.06	12.7
High	116.19	5.61	4.8	High	2.80	0.18	6.4
Mean c.v.			6.6	Mean c.v.			12.1

T4 data derived from 14 assays. T3 data derived from 12 assays.

range of 18.1 to 69 nmol/l for T4 and 1 to 2 nmol/l for T3. In both assays, recovery was excellent, mean \pm 1 s.d. (number of observations) being 106.0 ± 5.9 ($n = 11$) for T4 and 104.4 ± 8.5 ($n = 9$) for T3.

(c) *Precision and drift.* Precision was assessed by the inclusion of low, medium and high pools in each assay. The medium pool was prepared from normal feline serum and the low and high pools from this by dilution with hormone-free serum, and by addition of known amounts of T4 and T3 respectively. There was no evidence of drift as assessed by Student's paired *t* test on results from pools placed at the beginning and end of the assays (T4: $t = 0.03$, 16 d.f.; T3: $t = 0.50$, 25 d.f.). Within-assay c.v. for low, medium and high pools respectively were 5 per cent, 4 per cent and 2 per cent for T4 and 12 per cent, 5 per cent and 5 per cent for T3. Between-assay precision was excellent; the results are given in Table 2.

(d) *Detection limit (sensitivity) and working range.* Using the 4 parameter logit-log model for curve fitting, the calculated standard curve agreed with the observed standard points over the whole concentration range. The working range was therefore taken as being between the lowest and highest standards (T4, 5–200 nmol/l; T3, 0.125–10 nmol/l).

Serum total thyroid hormone concentrations in healthy cats

(a) *Distribution of values in healthy population* (all ages and both sexes). Both T4 and T3 values were found to fit a normal distribution (chi-squared goodness-of-fit tests on grouped data were not significant). Table 3 summarizes the data in terms of mean, s.d., range and 95 per cent range (2.5 per cent and 97.5 per cent sample percentiles).

(b) *Relationship between T4 and T3 levels* (all ages and both sexes). Individual T4 and T3 values were highly significantly correlated ($r = 0.46$, $P < 0.001$).

(c) *Effects of age, sex and breed on hormone levels.* Multiple regression was used to investigate the relationship of T4 and T3 levels to age, sex and breed. This

TABLE 3. Total serum T4 and T3 concentrations in healthy cats

Hormone	No. of Observations	Mean \pm 1 sd (nmol/l)	Range (nmol/l)	
			Actual	95%
T4	318	26.1 \pm 10.1	<5–54.1	8.5–46.2
T3	299	0.69 \pm 0.29	<0.13–1.48	0.13–1.27

TABLE 4. Multiple regression of age, sex and breed on total T4 and T3 levels in healthy cats. Results shown are t-statistics, testing significance of each factor for given levels of the others

Test	Results		Interpretation	
	T4	T3	T4	T3
Do total T4 or T3 concentrations change with age?	t = 3.74 (P < 0.001)	t = 4.14 (P < 0.001)	Highly significant relationship to age	Highly significant relationship to age
Do total T4 or T3 concentrations change with age in a quadratic (non-linear) manner?	t = 3.51 (P < 0.001)	t = 2.70 (P < 0.01)	Highly significant quadratic change	Significant quadratic change
Do total T4 or T3 concentrations of animals of a given age differ according to original sex?	t = 2.57 (P < 0.05)	t = 0.34 (NS)	Females show significantly higher levels than males at a given age	No significant difference between the sexes
Does castration or ovariectomy influence total T4 or T3 concentrations?	t = 0.62 (NS)	t = 0.47 (NS)	No significant effect when age is accounted for	No significant effect when age is accounted for
Does breed influence total T4 or T3 concentrations?	t = 1.17 (NS)	t = 3.53 (P < 0.001)	No significant effect of breed	Pedigree cats have significantly higher levels than domestic cats for any given age

NS = not significant

method tests whether or not mean hormone levels are significantly affected by each factor when the effect of all the others is taken into account. A quadratic term for age was included to test whether any relationship with age was linear. In view of the small numbers of pedigree cats, the analysis compared all pedigree to all domestic breeds. The former comprised 10 Siamese, 7 Burmese, 3 British blues, 2 Devon rex and 1 Persian, while there were 254 domestic short-haired and 24 domestic long-haired cats.

The results of the analyses as applied separately to T4 and T3 are summarised in Table 4. In the case of T4, both the linear and quadratic effects of age were highly significant and there was also a significant difference in T4 levels between the original sexes. Fig. 1 illustrates mean values (with standard error bars) for T4 for each sex and in five age groups, and shows that T4 levels in both sexes tended to decrease with age up to about 5 years and then to rise again. Female cats of a given age tended to have higher T4 values than male cats. The effects of neutering and breed were not significant for a given age and original sex.

For T3, a similar, though less pronounced, age effect was found, with the initial decrease with age being followed by a levelling out rather than an appreciable rise. The effects of original sex and neutering on T3 were not significant, but pedigree cats tended to have higher levels of the hormone at any given age than domestic cats (Table 4). Fig. 2 illustrates the differences in mean serum T3 concentrations of pedigree and domestic cats according to age. In neither case did Siamese cats

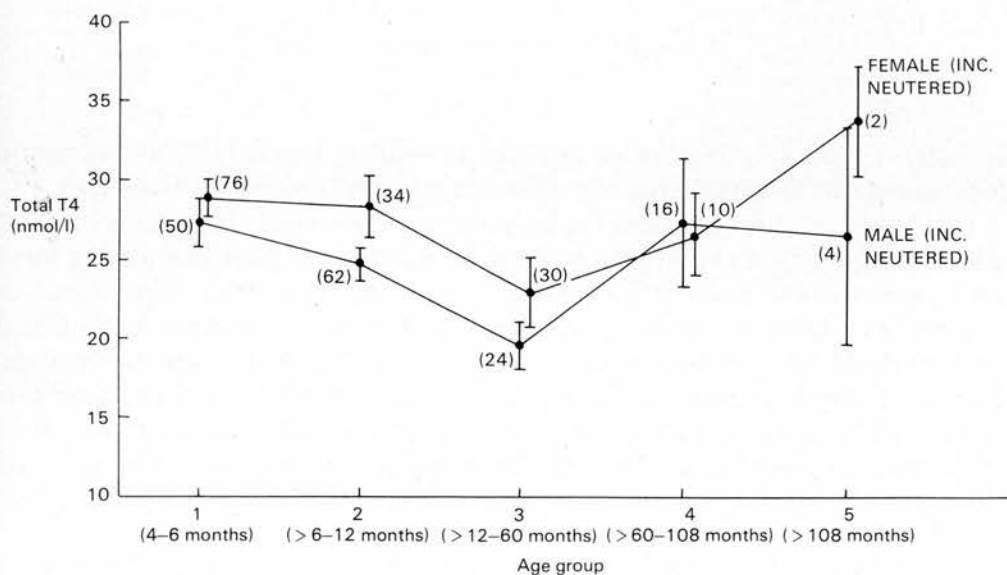


FIG. 1. Effects of sex and age on serum total T4 concentrations in healthy cats. The mean values (± 1 s.e.m.) are shown. The figures in parentheses are the number of observations in each group.

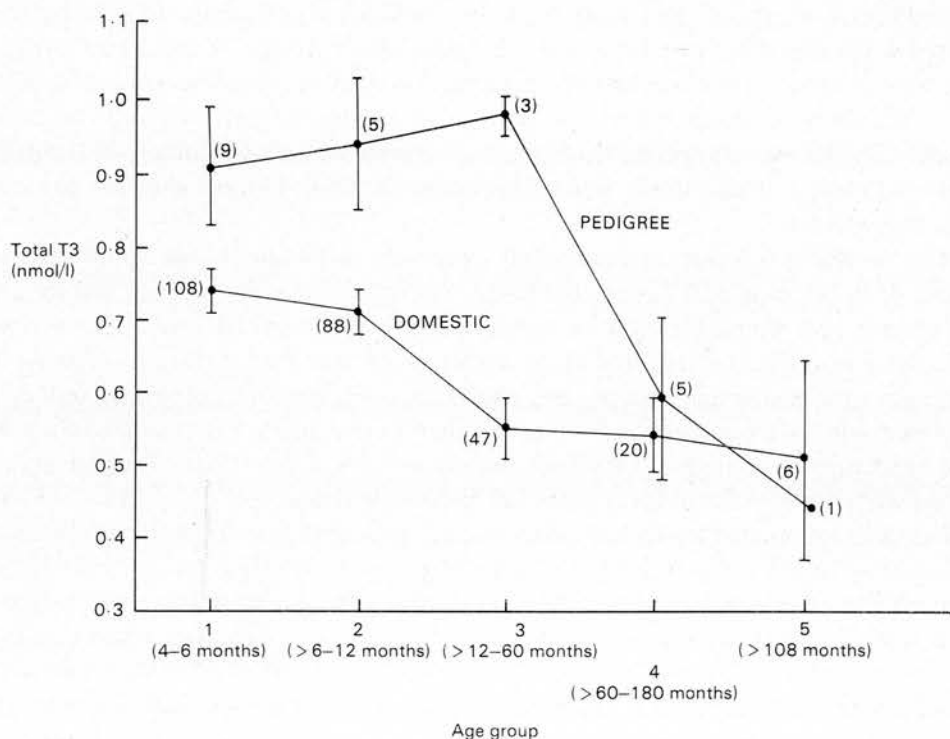


FIG. 2. Comparison of serum total T3 concentrations in pedigree and domestic cats according to age. The mean values (± 1 s.e.m.) are shown. The figures in parentheses are the number of observations in each group.

considered separately from other pedigree cats differ significantly from domestic long-haired cats for given levels of the other significant factors (see discussion).

(d) *Influence of heredity and environment on hormone levels.* If environmental effects influence T4 and T3 levels, it would be expected that cats reared in the same environment would show less variation in hormone levels than those reared in different environments. Similarly, a genetic component in hormone levels would lead to related cats varying less than unrelated ones. To examine these possibilities, hormone levels were measured in 13 unrelated cats grouped in five environments and in 18 related cats grouped in nine environments. Nested random-effect analysis of covariance was used to test for significant between-environment and between-family effects while correcting for the previously established effect of age on T4 and T3 levels.

The results of these analyses are shown in Table 5 together with their interpretation. The significant excess variation between environmental groups for the related cats suggests that hereditary and/or environmental influences exist on both T4 and T3, but it is not clear which component predominates for T4. For T3,

TABLE 5. Influence of environment and heredity on total T4 and T3 levels in healthy cats

Test	Results		Interpretation
	T4	T3	
Are there significant differences in T4 and T3 values of <i>related</i> cats in the same, compared with different environments? (6 and 9 d.f.)	$F = 10.09$ ($P < 0.01$)	$F = 5.51$ ($P < 0.05$)	Significant environmental <i>or</i> hereditary effect on T4 and T3
Are there significant differences in T4 and T3 values of <i>unrelated</i> cats in the same, compared with different, environments? (4 and 4 d.f.)	$F = 5.11$	$F = 3.48$	No evidence of significant environmental effects alone
Comparison of variation (within environments) for unrelated and related cats (4 and 9 d.f.)	$F = 2.17$	$F = 4.01$ ($P < 0.05$)	For T3 only, related cats in the same environment have significantly less variable values than unrelated animals, i.e. evidence for an hereditary effect

there appears to be a definite hereditary component, but these results on small numbers of animals clearly need to be confirmed by further studies.

DISCUSSION

Measurements of the circulating total T4 and total T3 by RIA are now widely used in the investigation of thyroid disease in man. Such techniques are applicable to the cat provided the methods are modified to allow for the much lower levels of circulating total T4 and T3 in this species compared with man.

Reap, Cass & Hightower (1978) and Anderson & Brown (1979) appear to be the only workers to have reported details of the RIA methods they used to determine plasma total T4 and total T3 levels in normal cats. The former workers measured total T4 and T3 in only 10 animals, whereas the latter evaluated total T4 but not T3 in 92. Both groups used commercially produced RIA kits which were designed for use with human serum. Both groups recognized the particular problems in using these kits to assay the low levels of thyroid hormones found and attempted to circumvent them by doubling the volume of serum used and halving the results to give the correct hormone concentration. Neither assay was optimized for the cat and standards were prepared in buffer. In a report on electrocardiographic changes in hyperthyroid cats, Peterson *et al.* (1982) employed a double antibody technique for T4 and T3 modified by using charcoal-extracted, pooled

serum from normal cats to produce T4 and T3 depleted serum for addition to control and standard tubes. Further details were not reported.

The assays described here were specifically designed for use with cat serum. The dilutions of anti-T4 and anti-T3 sera were adjusted to permit measurement of the relatively low concentrations of the hormone in cat serum. Correct standardization of the assays is essential and for this reason standards were prepared in resin-stripped cat serum which equalizes protein concentrations in standard and sample tubes and compensates for residual protein interference not eliminated by ANS. The stripping method adopted has been recommended for use with human serum as it does not affect the major thyroid hormone-binding protein concentrations (C. R. Salter, personal communication), whereas charcoal stripping as used by Peterson *et al.* (1982) tends to remove a proportion of such proteins. When used with cat serum, the technique showed no definite trends. Albumin levels were sometimes lowered but alpha-1 and alpha-2 globulin levels were not (human thyroxine-binding globulin migrates in the inter $\alpha 1/\alpha 2$ zone on electrophoresis). The procedure was efficient, removing greater than 99 per cent of T4 and T3, while providing a yield of approximately 70 per cent. It is generally accepted for man, that serum is satisfactory for standard production if greater than 95 per cent of the hormones have been removed.

The inclusion of the protein-binding inhibitor ANS in the incubates was found to be necessary despite reports in the literature that high affinity binding proteins for thyroid hormones could not be demonstrated in cat serum (Tanabe, Ishii & Tamaki, 1969; Scherzinger, Guzy & Lorcher, 1972; Bigler, 1976b). As barbital contributes to the blocking of the binding of thyroid hormones to their carrier proteins, it was thought that it might be possible to omit ANS from the incubate, particularly in the T3 assay. However, in the absence of ANS, T3 levels were consistently and incorrectly 20 per cent lower, and ANS was therefore included in both assays.

Data on within and between assay precision and on accuracy is essential if values for normal ranges are to be considered reliable. Reimers *et al.* (1981) are the only workers to have described validation of radioimmunoassays for T4 and T3 in cats, but normal ranges were not reported. They investigated solid phase kits designed for use with human serum and produced additional standards to allow measurement of the lower levels of the hormones found in normal cats. In the present study, within-assay precision for T4 and T3 and between-assay precision for T4 were excellent. Between assay precision was less good for T3 but compared well with that of similar assays and reflected the low concentrations of T3 measured.

This study, based on 318 T4 estimations and 299 T3 estimations is more comprehensive than any previously described (Table 6). The values reported here can be considered reliable as they were obtained with well validated methods on a large population. Although the mean T4 and T3 levels found here are in good agreement with previously reported values, it is evident that some euthyroid levels found in other series would be in the hyperthyroid range on the basis of the present data.

Three of the T4 and 10 of the T3 estimations (0.94 per cent and 3.35 per cent respectively) gave values that were less than the lowest standards of the assays. In each case, however, the paired T3 or T4 concentration was within the 95 per cent confidence limits for the respective assay. The assays were designed for the diagnosis of thyroid hypo and hyper-function and inevitably there will be some normal animals which will have hormone concentrations outwith the 95 per cent range. To have re-optimized the assays to measure the lowest levels of T4 and T3 found would have meant a loss in sensitivity at the higher end of the range. In addition, the values of the 2.5 per cent sample percentiles were greater than the lowest standard for each assay.

In this study, the serum T3 concentration was found to be highly significantly related to the serum total T4 concentration. While the methods of T3 production in the cat have not been established, such a relationship would be expected if its derivation is similar to that in man where only a small proportion of circulating T3 is secreted by the thyroid, approximately 80 per cent of it being produced from extra-thyroidal mono-de-iodination of T4 (Braverman & Vagenakis, 1979).

Both RIA and CPB techniques have been used to evaluate age differences in feline total T4 levels. Using RIA, Anderson & Brown (1979) found no significant age effects in 92 cats varying between 5 months and 15 years, but the actual numbers in each group were not stated. Using CPB methods, no age effects were noted by Ling, Lowenstine & Kaneko (1974) who evaluated 39 animals, but none was older than 5 years. Bigler (1976a), using a CPB technique, reported T4 levels in 110 cats. However, 92 were between 8.5 months and 1.5 years and only 4 were older than 4 years and the author was therefore unable to comment about age differences. Kallfelz & Erali (1973) evaluated the T4 levels of 5 cats aged 10 weeks, 4 aged 1 year and 5 that were more than 1 year old. There was a trend towards decreasing concentrations with increasing age but the results were not statistically significant.

In this study, total T4 levels were found to vary highly significantly with age in a non-linear manner, mean levels decreasing in both males and females until > 12–60 months and increasing thereafter. T3 levels also showed this non-linear decrease with age but tended to level out rather than increase at very high ages. Such data on T3 have not been previously reported. It would be expected that T3 changes would parallel those for T4 in view of the findings that T4 and T3 concentrations were highly correlated. In man, thyroid hormone levels decrease with increasing age in hospital patients, but not in the healthy elderly; that is, the decrease appears to be a consequence of illness rather than age *per se* (Olsen, Laurberg & Weike, 1978). Cats for inclusion in this study were taken from a healthy rather than a hospital population but, nevertheless, the rise of total serum T4 levels in older animals was not expected.

Sex differences in total T4 levels have been evaluated by both RIA and CPB techniques but total T3 levels only by RIA. Using RIA, Reap, Cass & Hightower (1978) found that there were no sex differences in total T4 and T3 levels but did not state whether any animals had been neutered. Anderson & Brown (1979) found no

TABLE 6. Reported levels of total T4 and T3 by radioimmunoassay techniques in healthy cats

Reference	Method	No. of cats	T4 (nmol/l)		T3 (nmol/l)	
			Range	Mean \pm 1 sd	Range	Mean \pm 1 sd
Reap, Cass & Hightower (1978)	NR	10	15.2-38.0	26.0 \pm 7.9	0.60-1.73	1.00 \pm 0.32
Anderson & Brown (1979)	Solid phase	92	18.0-83.7	39.8 \pm 24.5	ND	ND
Peterson, Johnson & Andrews (1979)	NR	NR	18.0-51.5	NR	1.16-3.08	NR
Holzworth <i>et al.</i> (1980)	NR	NR	19.3-64.4	NR	0.92-3.08	NR
Theran & Holzworth (1980)						
Peterson, Becker & Hurley (1980)	NR	NR	NR	27.0 \pm 6.4	NR	0.41 \pm 0.19
Peterson <i>et al.</i> (1981)						
Jones & Johnstone (1981)	Polyethylene glycol separation	NR	30.0-50.0	NR	ND	ND
Peterson (1981)	NR	NR	12.9-38.7	NR	0.39-1.54	NR
McMillan & Scherding (1981)	NR	NR	12.9-51.5	NR	0.92-3.02	NR
Nesbitt (1981) quoted by Rosychuk (1982)	NR	NR	NR	28.3 \pm 7.7	ND	ND
Peterson (1981) quoted by Rosychuk (1982)	NR	NR	ND	ND	NR	0.49 \pm 0.22
Theran (1981)	NR	NR	12.9-51.5	NR	0.92-3.08	NR
Watson <i>et al.</i> (1981)	NR	3	23.0-38.0	NR	ND	ND
Hoenig <i>et al.</i> (1982)	Double antibody	T4-51 T3-44	9.0-45.1	28.3 \pm 7.7	0.20-1.17	0.49 \pm 0.22
Peterson <i>et al.</i> (1982)	Double antibody	NR	16.7-42.5	NR	0.23-0.77	NR

TABLE 6 (continued)

Reference	Method	No. of cats	T4 (nmol/l)		T3 (nmol/l)	
			Range	Mean \pm 1 sd	Range	Mean \pm 1 sd
Reimers (1982)	NR	NR	9.7-56.3	NR	0.23-1.23	NR
Belshaw (1983)	NR	NR	19.3-45.1	NR	0.77-2.31	NR
Kirk (1983)	NR	NR	14.1-48.9	NR	NR	NR
Noxon <i>et al.</i> (1983)	NR	NR	19.3-51.5	NR	ND	ND
Peterson <i>et al.</i> (1983)	Double antibody	67	10.3-45.1	27.0 \pm 10.5	0.23-0.92	0.51 \pm 0.25
Peterson <i>et al.</i> (1983)	Solid phase	135	10.3-48.9	27.0 \pm 15.0	0.23-1.60	0.81 \pm 0.32
Peterson & Becker (1984)						
Peterson & Yoshioka (1983)	NS	NS	19.3-64.4	NR	0.92-3.08	NR
Present study	Double antibody	T4- 318 T3- 299	<5.0-54.1	26.1 \pm 10.1	<0.13-1.48	0.9 \pm 0.29

NR = not reported. ND = not determined

significant differences between T4 levels of male and female cats but entire females had significantly higher levels than those that had been ovariectomised. Using CPB, Ling, Lowenstine & Kaneko (1974) also found that the sex of cats did not affect circulating total T4 levels. Bigler (1976a), however, reported that female cats had significantly higher T4 levels than males. In man, thyroid hormone levels have been reported to be slightly higher in females than in males, but there is no universal agreement on this. Indeed, the tendency to higher levels in females may have been due to the inadvertent inclusion of some pregnant women in the series (Robbins & Rall, 1979).

In this study, an attempt was made to exclude pregnant animals by physical examination. For any given age, female and neutered female cats taken as one group, tended to have significantly higher T4 levels than males and neutered males. No such effect was seen with T3. When age was accounted for, the effects of castration or ovariectomy were not significant for either T4 or T3.

In the only report on breed differences in thyroid hormone levels, Ling, Lowenstine & Kaneko (1974), using CPB, found a significantly higher level of total T4 in Siamese ($n = 11$) compared with domestic long-haired cats ($n = 7$) but no difference between T4 in either of these breeds and domestic short-haired cats ($n = 10$). Because our study only included 23 pure-bred cats, data were pooled and compared with those for domestic short- and long-haired animals taken together. No significant differences in total T4 levels were found but the total T3 values were significantly higher for any given age in the pedigree breeds. When the Siamese breed was compared separately with domestic long-haired cats, T4 and T3 levels did not differ significantly.

There was an apparent similarity in both T4 and T3 values respectively of animals kept in the same environment and this was proven statistically. However, when the animals were separated into related and non-related groups, it was not possible to show which component predominated for T4. For T3 there appeared to be a definite genetic component. Such findings have not been reported previously but they require further studies in larger numbers of animals.

This paper describes the first RIA specifically designed for measurement of thyroid hormones in cat serum and details the steps required for its optimization. It is the largest study so far conducted and presents data not previously reported on possible variables which, it is hoped, will be of use to both clinicians and research workers.

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